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TECHNICAL MANUSCRIPT 544

ELECTROPHORESIS OF COLLOIDAL BIOLOGICAL PARTICLES

John F. Lemp, Jr.
Eugene D. Asbury
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AUGUST 1969

DEPARTMENT OF THE ARMY
Fort Detrick
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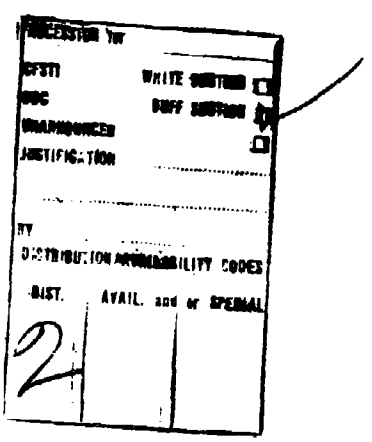

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John F. Lemp, Jr.

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Process Development Division
AGENT DEVELOPMENT & ENGINEERING LABORATORIES

Project 1B562602A082

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ABSTRACT

Biological particles in liquid suspension exhibit varied electrophoretic mobility dependent upon the electric charge behavior of their surface constituents. The surface composition of a population of the same kind of biological particles is uniform in a constant environment. The microscope electrophoresis techniques for mobility and isoelectric point determinations of microscopic particles (bacteria, suspended mammalian tissue cells, aluminum oxide particles, and polystyrene latex particles) and submicroscopic particles (proteins and gelatin) are described. The information that can be obtained and the additives for modification of electrophoretic mobility determined by the analytical method are applied to the preparative, laminar-flow, continuous particle electrophoresis system. Separation of bacteria and bacteriophage from suspensions by continuous particle electrophoresis is described.

DIGEST

Microscope electrophoresis was used to measure the electrophoretic mobility of aluminum oxide and polystyrene latex particles, bacterial, and mammalian tissue cells. The submicroscopic hydrophilic colloids (gelatin, serum albumin, and staphylococcal enterotoxin B) were adsorbed on latex carrier particles to determine their electrophoretic mobility and the effect of concentration, pH, electrolyte addition, and buffer ionic strength.

Mobility curves as a function of pH were established for latex particles at 1 ppm concentration indicating an isoelectric point (IEP) at pH 3.6. The IEP for Escherichia coli B cells was measured at pH 2.8, Serratia marcescens at pH 2.6, Bacillus subtilis var. niger at pH 2.9, and L strain mouse fibroblast cells at pH 4.4. Using an adsorption technique, isoelectric points were measured for proteins: gelatin (acid form) at pH 9.4, serum albumin at pH 4.9, and staphylococcal enterotoxin B at pH 6.3.

Procedures for examining electrophoretic characteristics of microscopic and submicroscopic biological particles are described in order to standardize procedures and to generate results applicable to an understanding of parameters influencing concentration and purification of colloidal biological particles.

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I. INTRODUCTION*

The two initial purposes of the work reported here were (i) to gain a better understanding of methodology and (ii) to determine the effects of pH, electrolyte addition, particle concentration, ionic strength and sample preparation on the electrophoretic migration of colloids in liquid suspension. Our ultimate goal was to determine if electrophoretic mobility measurement and control could be used to enhance the collection, concentration and purification of colloidal biological particles.

Electrophoretic mobility (EM) is defined as the velocity of particle migration (in microns per second) in an electric field with a potential gradient of 1 volt per centimeter, expressed as (μ/sec)/(v/cm). The specific conductance (SC) of the fluid suspension, expressed as micromhos per centimeter (μmhos/cm), may be measured along with EM. Zeta-potential (ZP), the potential difference between the surface of shear of the thin layer of liquid fixed to the wall of a colloidal particle and the bulk of the suspending fluid, may be calculated from EM data. Because many colloid chemists consider the calculated ZP an oversimplification and subject to error, we express our results as EM, a measured value.

There are three general methods for determining electrophoretic mobility of colloids: (i) moving-boundary electrophoresis, (ii) zone electrophoresis, and (iii) microscope electrophoresis. Moving-boundary electrophoresis measures the velocity of a mass of particles by following the movement of the particulate boundary as influenced by a voltage gradient and requires a relatively large sample for measurement; Alberty[1] and Longsworth[2,3] have reviewed the essentials of this method. Zone electrophoresis is, in fact, a combination of electrophoresis and chromatography or solid medium electrophoresis; this method is reviewed by Block, Durrum, and Zweig,[4] Wunderly,[5] and Kunkel and Trautman.[6] Microscope electrophoresis is the direct microscopic tracking of individual particles as they migrate under the influence of an electrical field; this method is reviewed by Brinton and Lauffer[7] and detailed by Riddick.[8]

The advantages of the microelectrophoretic method include: small concentrations of particles can be measured; the shape, size, and orientation of the particles may be observed; relative mobilities can be determined by observing the migrations of individual particles; and, in general, the equipment is inexpensive and easy to maintain. The disadvantages are the difficulty in adapting the procedure for preparative purposes, and the limitation imposed by the microscope's resolving power, which makes it impossible to observe and measure the action of submicroscopic particles unless they are adsorbed on carrier particles of observable size. In the

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adsorbate-carrier system, the electrophoretic properties of the carrier particles are wholly masked by the adsorbed colloid film; however, any contaminant in the adsorbate may very well influence the properties of migration just as any microscopically visible, colloid system may be influenced by a contaminating film of impurity, which is electrophoretically mobile.

The electrophoretic mobility measured for submicroscopic colloids adsorbed on carrier particles must be verified by a preparative technique so that the purity may be determined chemically or biologically. Because the microscope electrophoresis method was to be a basis for a preparative technique, we preferred a preparative device employing free electrophoresis because the colloid fraction could be separated directly from the suspended sample of the biocolloid rather than indirectly via paper, acrylamide gel, or starch gel or by elution from a packed column. Thus, we used a continuous particle electrophoresis apparatus, which is a laminar-film, continuous-flow instrument that provides purified colloid fractions at a rate of milliliters per hour.

Because we were primarily interested in colloidal biological materials and systems, microscope electrophoresis was selected and used for the analytical method, and continuous particle electrophoresis was selected and used for the preparative method. We observed the colloid either directly or adsorbed on carrier particles. The colloid was resuspended in a suitable buffer from its native menstruum. If the colloid was an intact cell, the viability was preserved during electrophoretic studies by resuspension in an isotonic buffer at a suitable pH.

II. THEORY

In many instances, optimum flocculation of colloidal, biological material occurs at near zero EM, and maximum dispersion occurs at maximum mobility. Some biocolloids may be soluble at their isoelectric point (IEP), but they are most easily affected by oppositely charged polyions at this point.[9] Laboratory measurement and control of the EM of biological colloidal suspensions during processing may well improve the efficiency of the rate methods of separation (sedimentation and electrophoresis), as well as the equilibrium methods of separation (dialysis, filtration, adsorption, and liquid-phase extraction).

Biocolloids represent colloid systems with reversible changes of state and electrolytic characteristics. These systems are often called hydrophilic, lyophilic, reversible, and resoluble and were previously called emulsoids (includes proteins and higher carbohydrates, as well as bacterial and viral suspensions). The Maxwellian free energy, F, of the lyophilic dispersed system has a lower value than the system divided into phases; i.e., $F = U - (TS)$, where U is the internal energy, T is the absolute temperature, and S is entropy. Reversible colloid systems generally require large changes in conditions to revert to the original nondispersed state and, hence, involve considerable increases of entropy. This is not different from true solutions because the increase in entropy from the dispersion promotes a decrease in free energy. The lyophilic colloid is present in true solution. The lyophilic state represents an equilibrium state and can be reached spontaneously, starting from the dry colloid and an appropriate solvent.[10] The electrolytic character of the sol furnishes a further stability factor because the dispersion will continue to exist (and the colloid to migrate electrophoretically) if the particles carry an electric charge. The reduction of the electrical charge leads to flocculation. Certain reagents decrease the solubility of colloids by changing the character of the electric double layer surrounding all particles in liquid suspension. This effect occurs typically in globulin proteins, which are disperse systems except at their isoelectric point (where there is no charge or where the charges are at equilibrium); thus, there is zero ZP and zero EM.

The biological particles are likely to act much as protein because of their chemical makeup. Proteins are amphoteric: in acid solutions, they become a cation or a dipolar cation; near neutrality, a dipolar isoion defining the isoelectric point; and in basic solution, a dipolar anion or an anion. A reversal of charge is associated with pH, and the point at which the reversal occurs is dependent on the make-up of the particle or protein in question. Likewise, the electrokinetic charge, which is of the same order of magnitude as the titrated charge (binding of H^+ and OH^- ions), occurs in colloidal proteins and shows a similar dependence on pH and on ionic strength. Thus, flocculation of colloids can also be caused by concentration of the colloid, addition of electrolyte or counterions, or even, as Lauffer[11] suggests, by the addition of competing like-charged ions.

The variation of EM among different viable biological particles depends on the chemical constituents of the surface, which are amazingly uniform within a species population. The surface charge behavior of viruses is generally the same as that of proteins; the surface charges of bacterial cells can correspond with one, or a combination, of the behaviors of proteins, lipids, or polysaccharides.

III. MATERIALS AND METHODS

A. MICROSCOPE ELECTROPHORESIS APPARATUS

The microscope electrophoresis apparatus used for these experiments employs the Tyndall effect to reflect flashes of light from individual particles in liquid suspension into a stereoscopic microscope, where movement of the particulates, as affected by an applied electric potential, can be followed and their migration velocity timed. The microscope does not make the particles visible in the usual sense but only makes them perceptible as pinpoints of light; this greatly lowers the minimum observable contrast and enables the particle migration to be seen. The apparatus can be used to measure both EM and SC.

A complete description of this equipment* is available[8] and only a cursory description is necessary here. The apparatus is quite similar to apparatus previously described by various investigators[7,12-14] and consists of:

1) A continuously variable (0 to 500 volts) d-c power supply with a reversible polarity switch and a precision voltmeter and micro-ammeter.

2) An electrical timer accurate to tenths of a second with a fast-acting contact switch.

3) A clear plastic electrophoresis cell with plastic sol chambers containing platinum-iridium electrodes positioned 5.0 cm on either side of the center of a polished, cylindrical cell tube (4.4 mm inside diameter) that connects the two sol chambers (Fig. 1). A molybdenum anode is employed with high-specific-conductance systems.

4) A heat-absorbing cell holder and mirror arrangement, which reflects a beam of light at an angle through the cell tube to the stereoscopic microscope, is used to view and measure the rate of travel of discrete particle images through the cell tube.

A simple modification to the basic cell allows cumulative additions and mixing of additives without removing the cell or replacing its contents. The addition of approximately 15 inches of 0.25-inch-internal-diameter clear tubing to the sealed left cell chamber electrode and thence to a bulb pipette filler allows cumulative reagent additions to the right cell chamber with mixing by alternate siphoning and release of the cell chamber contents; the tubing serves as a mixing reservoir. After thorough mixing, the tube is clamped near the left electrode to prevent migration of the fluid material, by convection and external pressure gradients, within the cell tube during measurement.

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* Zeta Meter, Inc., 1720 First Ave., New York, New York 10028.

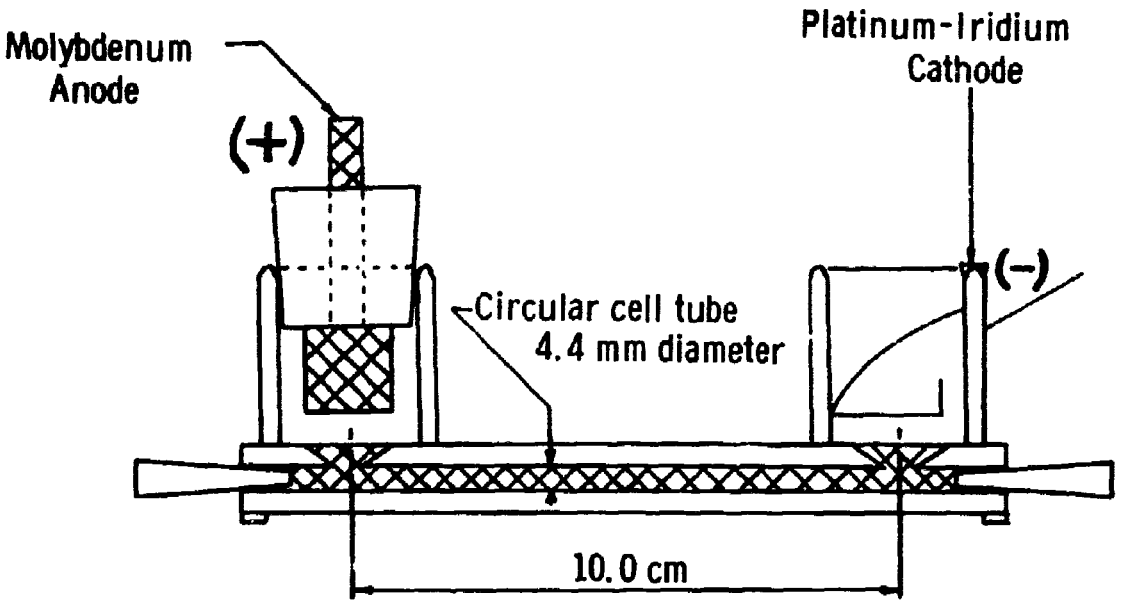

FIGURE 1. Cross-Section of Microscope Electrophoresis Cell.

The rate of electromigration was found by cumulatively timing 20 to 50 single-particle transits over the microscope graticule at an applied potential of approximately 20 v/cm. The average migration was computed by dividing the cumulative migration time by the total number of ocular micrometer spaces traversed to obtain the average time for traverse of one micrometer division.* The EM was then obtained by curves based on the voltage employed, the micrscope magnification, and the micrometer length, with temperature corrected to 25 C.

A sample transfer system, using a syringe pumping system to fill and transfer material to and from the cell and an agitated pH reservoir, was adapted for determining pH versus mobility curves.

* This timing method was suggested by Riddick.[6] Reversed polarity, frequently used for average EM measurement, is not recommended with the platinum-iridium electrodes or with the molybdenum, high-specific-conductance electrode with this cell.

B. COLLOID CARRIER PARTICLES

Overbeek and Bungenberg de Jong[15] indicated that finely divided, but microscopically visible, hydrophobic particles (quartz, carbon, oil drops, aluminum oxide, and silica gel), when completely covered by a hydrophilic colloid film, are wholly masked in their electrophoretic properties by the colloidal additive; thus, the hydrophobic particle assumes the properties of the added hydrophilic film surface. Abramson[16] and Abramson, Moyer, and Gorin[14] stated that migration of like particles coated with a protein colloid film is independent of the size of the carrier particles. This knowledge allows the selection of a microscopic carrier particle for readily observing electromigration velocities as influenced by the hydrophilic coating on the particle surface. Aluminum oxide particles of uniform size, 0.3 μ,* were first used for test purposes.

James[17] suggested that uniformly sized latex beads might serve as a reference and comparison carrier standard for microelectrophoretic systems. This suggestion led to further consideration of latex spheres as a colloid carrier for various macromolecules adsorbed as a film on the carrier surface. Polystyrene latex particles** 1.099 μ in diameter, with a standard deviation of 0.0059 μ, were used for these characterization studies.

C. TRYPTOSE PHOSPHATE MEDIUM

Dehydrated tryptose phosphate broth (Difco) was resuspended 29.5 g in 1,000 ml of deionized water and sterilized for 15 minutes at 15 pounds pressure (121 C).

D. E. COLI PHAGE, T-3 LYSATE

Sterile tryptose phosphate broth was inoculated with Escherichia coli B culture, 0.1% (v/v) of 1×10^8 to 2×10^8 viable cells per ml. The inoculated medium was agitated and incubated at 37 C for 2½ hours. Between 1 and 5 multiplicities of infection (MOI) of T-3 phage were added, and the culture was incubated for another 2½ hours; lysis of the culture was apparent. Cells were removed by filtration through a Mandler filter of normal porosity into a sterile flask for storage at 4 C; phage lysate generally obtained 1×10^{10} to 2×10^{10} plaque-forming units per ml (PFU/ml). Phage assays were made by the agar layer technique described by Adams.[18]

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* Will Scientific, Inc., Box 5195, Baltimore, Maryland, 21224.

** Diagnostic Products, The Dow Chemical Co., PO Box 512, Midland, Michigan. Supplied as 10% solids with a density of 1.05 g/cc and a refraction index of 1.592 at 20 C measured at 5,400 Å. Latex particles are available from 0.088 through 1.099 μ in diameter.

E. DEIONIZED WATER DILUENT

Deionized water, with enough 0.02 M K_2HPO_4 added to adjust current flow through the diluent to 10 μ amp when measured at 200 v, was used as indicated.

F. IMIDAZOLE BUFFER

Six grams of imidazole was added per liter of deionized water. The ionic strength was calculated at 1.3×10^{-4} at pH 8.6. This diluent was further diluted 1:100 for diluting or washing cell cultures. Sucrose at 0.32 M was added as indicated.

G. TISSUE CELLS

Earle's strain mouse fibroblast cells were grown in suspension* for eight days with the pH controlled at 6.9 and oxidation-reduction potential (ORP) controlled at 50 millivolts (mv). Culture assayed at 2.5×10^6 cells/ml, 82% viable, and 12.7 μ diameter average cell size. Cells were centrifuged at 2,500 rpm for 5 minutes, the supernatant fluid was decanted, and cells were resuspended in imidazole-sucrose diluent and recentrifuged through four washes. A small amount of packed cells from each wash was resuspended in imidazole-sucrose buffer for electrophoretic measurements.

H. BACTERIAL CELL CULTURES

E. coli was grown in tryptose-phosphate broth (Difco) with agitation at 37 C for 18 hours. Bacillus subtilis var. niger and Serratia marcescens cultures were grown in nutrient broth (Difco) with agitation at 37 C for 18 hours. Cells were concentrated by centrifugation in a Servall RC-2** centrifuge at 7,500 rpm (6,780 x g) for 10 minutes, the supernatant was decanted, and cells were resuspended in imidazole buffer or deionized water diluent through four washes. A small amount of the packed cells was resuspended in imidazole buffer or water diluent for electrophoretic measurement. Staphylococcus aureus cells, strain 10-275, derived from strain S-6, were grown in N-Z-Amine A medium*** in submerged culture; cells were concentrated by centrifugation and resuspended in imidazole buffer through two washes, with cells finally resuspended in imidazole buffer for mobility measurement.

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* Cells were provided through the courtesy of W.F. Daniels, Fort Detrick, and were grown in his standard medium (Earle BBS, modified, containing 10% bovine serum; Eagle MEM concentrates; lactalbumin hydrolysate; and cysteine-ascorbic acid without phenol red).

** Ivan Sorvall, Inc., Norwalk, Conn.

*** Medium composed of 5.0% N-Z-Amine A, 0.4% BYF-100 yeast, and 0.13% potassium acid phosphate ($K_2HPO_4 \cdot 2H_2O$) at pH 7.0.

I. CONTINUOUS PARTICLE ELECTROPHORESIS

The preparative, laminar-flow continuous particle electrophoresis device* (CPED) is quite similar to the microscope electrophoresis cell, except that it is possible to sample the mobile components during operation. The CPED is depicted in Figure 2 and has been fully described.[19-22] To operate the CPED, a low-ionic-strength buffer is prepared, and the two buffer reservoirs are filled. One buffer provides the laminar curtain within the cell; the other buffer portion is circulated counter-currently past the electrodes to remove any electrolysis products. The electrodes are separated from the curtain buffer by cellophane membranes. Once the curtain flow is established and the cell is completely full of liquid and free of air bubbles, the sample colloid is introduced (suitably diluted in the same buffer) from a variable-flow syringe pump. Note that the sample inlet tube is on the cathode side of the top center of the cell; this allows for the maximum horizontal migration of the normally electronegative biocolloids.

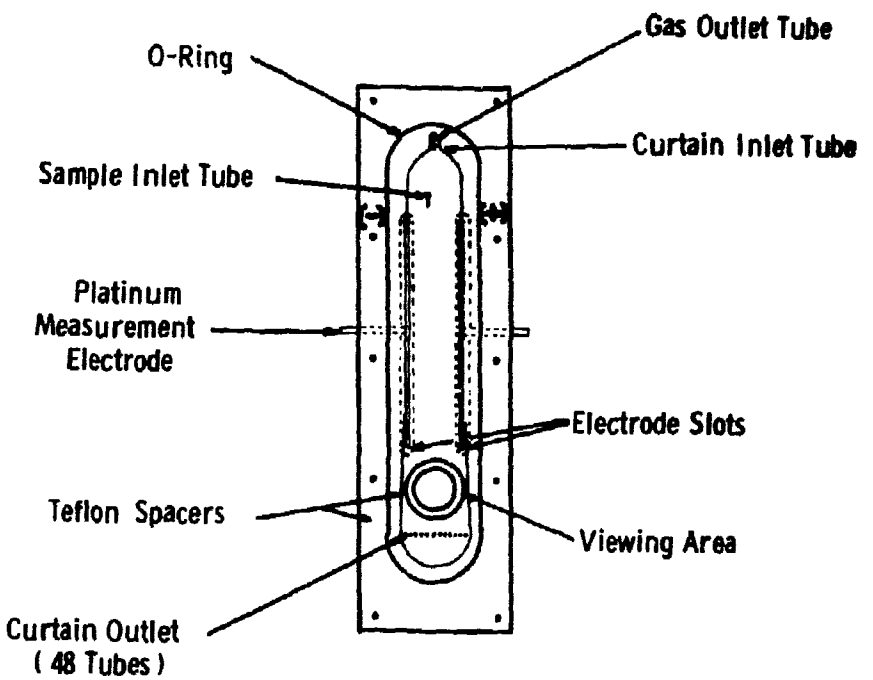

FIGURE 2. Continuous-Flow Particle Electrophoresis Cell.

* Beckman Instruments Inc., Scientific and Process Instruments Div., Fullerton, California, 92634.

The sample band is first established without applying voltage, this band position is noted in the fraction viewing area, and the control samples are obtained from the 48-tube fraction collector. The separating voltage gradient may then be adjusted between 10 to 100 v/cm; this control automatically maintains a constant voltage gradient by an additional pair of sensing electrodes in the cell. Because a horizontal d-c field is applied to the curtain buffer and sample, the particles have two components of motion: vertical (equal to the buffer velocity) and horizontal (proportional to the electrophoretic mobility). The visible band moves horizontally across the view area from its zero voltage control position. After the band position has stabilized, the mobile components may then be collected from the 48-tube fraction collector. Each tube in the collector at the bottom of the cell collects a 1-mm slice of the buffer-sample curtain.

The horizontal velocity of the particle band within the CPED may be calculated using the cell dimensions, the vertical flow rate of curtain buffer, the height of the electrodes, and the horizontal displacement of the visible particle band. This horizontal velocity in μ/sec divided by the applied potential gradient in v/cm provides the electrophoretic mobility of the particles.

The sharpness of the particle band within the CPED is dependent on: (i) sample flow rate, (ii) ionic strength and flow rate of the curtain buffer, (iii) applied voltage, and (iv) the temperature of the curtain buffer. Because of the short transit time for the sample in the laminar-flow cell, diffusion of the band of particles is considered negligible.

Preliminary results indicate that operational conditions should be standardized as follows: (i) 0.04 ml/min sample flow, (ii) 10^{-6} M imidazole curtain buffer at 20 ml/min flow, (iii) generally less than 40 v/cm of applied voltage, and (iv) the laminar-flow cell cooled to several degrees C below the buffer temperature to prevent bubbles from forming in the cell chamber and to minimize thermal overturn.

IV. RESULTS AND DISCUSSION

The experiments described in this report were designed to establish microscope electrophoresis methods for microscopic and submicroscopic biological particles. For the submicroscopic biological particles, the selection of an appropriate colloid-carrier particle was required.

The microscope electrophoresis methods were to be tested with selected microscopic and submicroscopic biological particles. The electrophoretic mobilities of the biological particles were to be modified by changes in pH and by additions of polyelectrolytes in order to improve separation when applied to sedimentation, flocculation, and filtration unit operations.

Finally, the microscope electrophoretic mobilities of the biological particles were to be used to predict separations by continuous-flow particle electrophoresis, and these separations were then to be demonstrated.

All experimental results comprise the data from at least four replications; the EM values are replicable within ±0.5 (μ/sec)/(v/cm) and the isoelectric point (IEP) values are replicable within ±0.2 pH units.

A. ELECTROPHORETIC CHARACTERISTICS OF ALUMINUM OXIDE CARRIER PARTICLES

Aluminum oxide (Al_2O_3) particles (0.3 μ) were added to deionized water diluent (pH 6.2) in the microscope electrophoresis cell in increasing concentration, with the results shown in Figure 3. A reversal of migration from electronegative to electropositive is indicated between 200 and 300 ppm aluminum oxide. This concentration phenomenon allows a choice of either an anionic (<200 ppm) or a cationic (>300 ppm), hydrophobic colloidal-carrier particle system.

Al_2O_3 particles at 100 and 1,000 ppm, when adjusted to various pH levels with dilute hydrochloric acid or sodium hydroxide, migrate as shown in Figure 4. The cationic (1,000 ppm) system reverses from positive to negative migration above pH 6.6;* the anionic system (100 ppm) maintains its characteristic negative mobility between pH 4.0 and 8.0.

Addition of 1% (v/v) of either nutrient medium, tryptose-phosphate medium, a 5% sodium chloride solution, or *E. coli* T-3 phage lysate (tryptose-phosphate medium containing 2×10^{10} PFU/ml) to three concentrations of Al_2O_3 particles in deionized water gave the results shown in Table 1. No reversal of Al_2O_3 mobility occurred when either of the media or phage lysate were added, indicating that these hydrophilic colloidal additives imposed their own characteristic anionic charges on the carrier particles.

* Care must be taken to insure the carrier system is defined for pH, otherwise a reversal may occur as a function of pH rather than as a function of particle concentration.

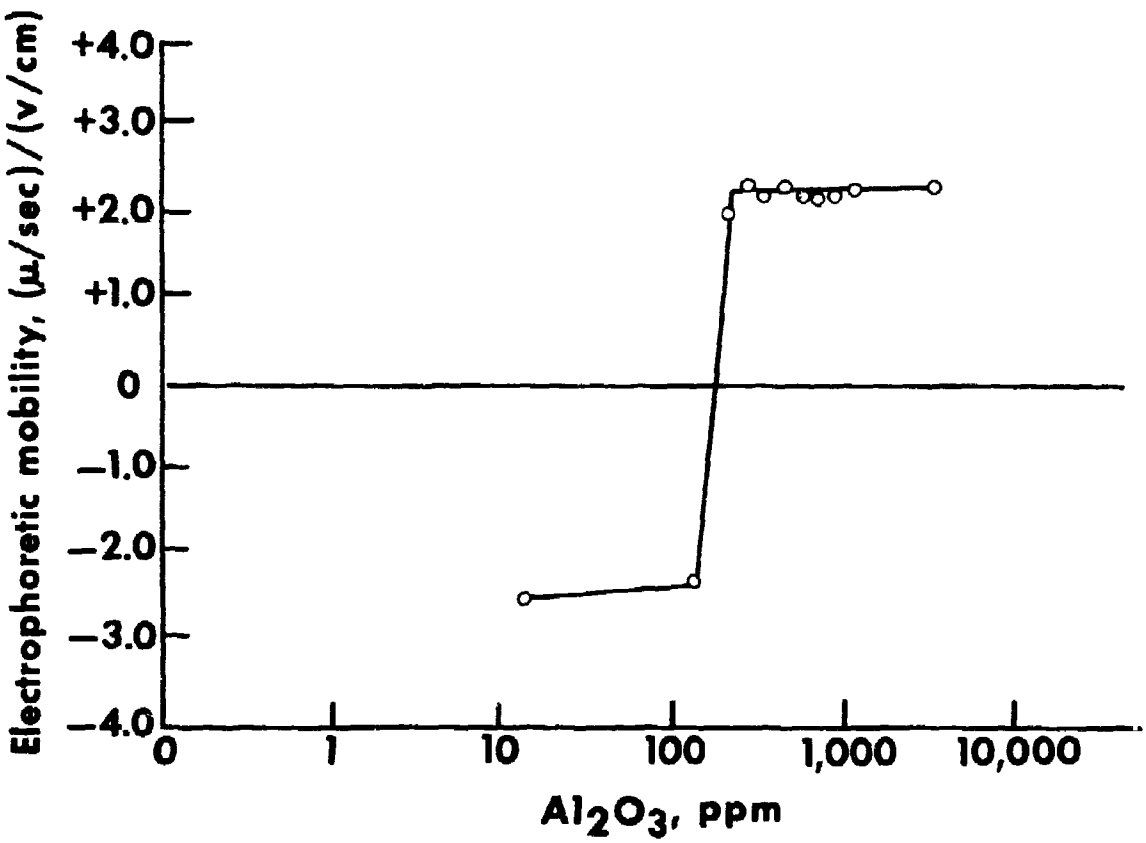

FIGURE 3. Reversal of Migration of Hydrophobic, Colloid Carrier Particles (Al_2O_3) as a Function of Increasing Concentration.

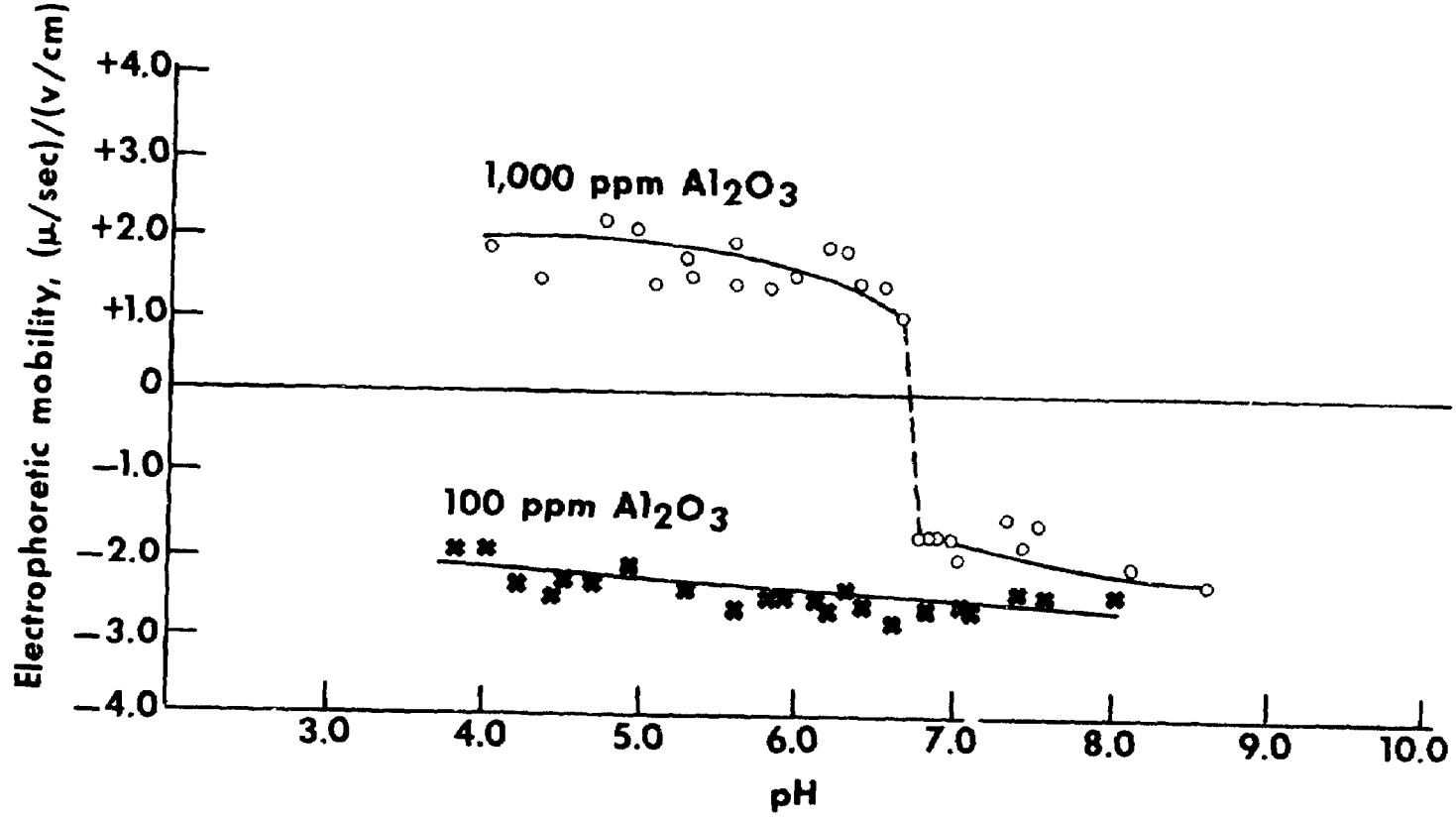

FIGURE 4. The Effect of pH on the Mobilities of Two Concentrations of Hydrophobic, Colloid Carrier Particles.

TABLE 1. ELECTROPHORETIC MOBILITY OF Al_2O_3 COLLOIDAL-CARRIER PARTICLES AS AFFECTED BY INCREASING CONCENTRATION AND BY FOUR ADDITIVES

Al_2O_3 Carrier, ppm	Electrophoretic Mobility, (μ/sec)/(v/cm)				
		After Addition of 1% (v/v) of:			
	Control[a/]	Nutrient Medium	Tryptose-Phosphate Medium	5% NaCl Solution	*E. coli* T-3 Phage Lysate
10	-1.8	-2.1	-2.2	-2.2	-2.4
100	-2.0	-1.9	-2.0	-2.1	-1.0
1,000	+3.1	-2.3	-1.3	+2.7	-2.1

a. Indicated concentration of Al_2O_3 in deionized water.

Because the direction of migration could be controlled as a function of concentration of Al_2O_3 carrier to produce either an anionic or a cationic system, a reversal of migration should be produced by the addition of oppositely charged polyionic additives. Therefore, a 2% solution (v/v) of cationic Primafloc C-3* was added to an anionic (100 ppm) Al_2O_3 carrier system, and a 1% solution (v/v) of anionic Benax 2A1** was added to a cationic (1,000 ppm) Al_2O_3 carrier system with the results shown in Figure 5. A reversal of migration occurred in both systems as predicted.

The extent of adsorption of a hydrophilic colloid is a function of adsorbate concentration, and a saturation value is reached at a concentration beyond which mobility is no longer affected. Tryptose-phosphate medium was added in increasing concentration to a colloid carrier suspension of 1,000 ppm Al_2O_3 particles, pH 6.2. The concentration plot indicates that migration was reversed before the growth medium concentration reached 1,000 ppm, and thermal overturn, due to high conductivity, occurred when the growth medium exceeded 10,000 ppm (Fig. 5). The reversal of migration again indicated that the electronegative peptone additive imposes its own electomobility on the normally electropositive carrier particle system as a function of increasing concentration.

Al_2O_3 (1,000 ppm in deionized water) was coated by a 1% (v/v) addition of tryptose-phosphate medium, thereby effectively reversing the EM at pH 6.2. The pH of this system was adjusted with dilute hydrochloric acid and with dilute sodium hydroxide. The electrophoretic characteristics were measured as function of pH as shown in Figure 6. The IEP of the tryptose-phosphate medium was observed at pH 4.3; below this pH, particles migrated

* Water-soluble, alkaline polyamine, Rohm & Haas Co., Independence Mall West, Philadelphia, Pa., 19105.

** Sodium salt of dodecyldiphenyl ether disulfonic acid, The Dow Chemical Co., Albott Road Building, Midland, Michigan, 48640.

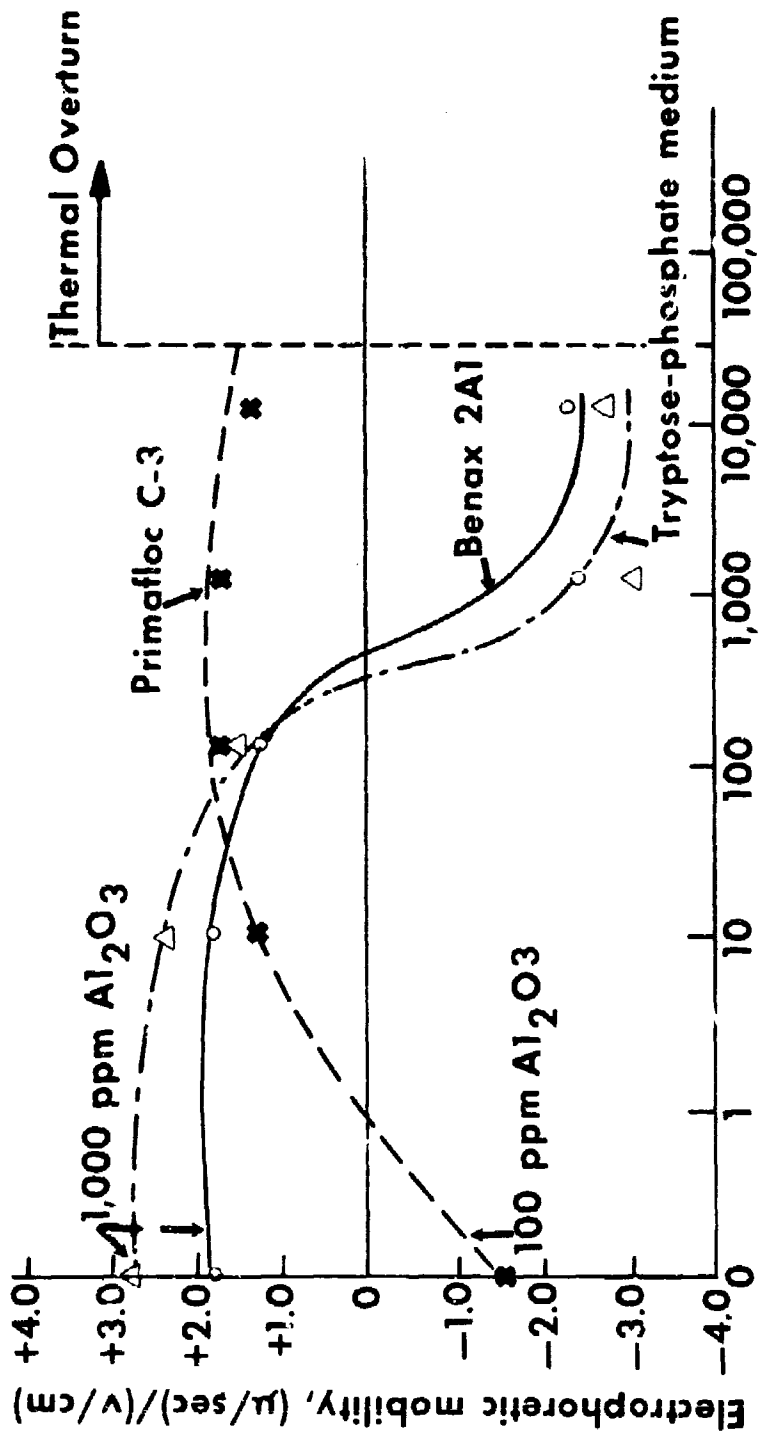

FIGURE 5. Reversal of Migration of Anionic (100 ppm) and Cationic (1,000 ppm) Hydrophobic Colloidal Carrier Particles (Al_2O_3) as a Function of Increasing Concentrations of Two Polyelectrolytes and Culture Medium.

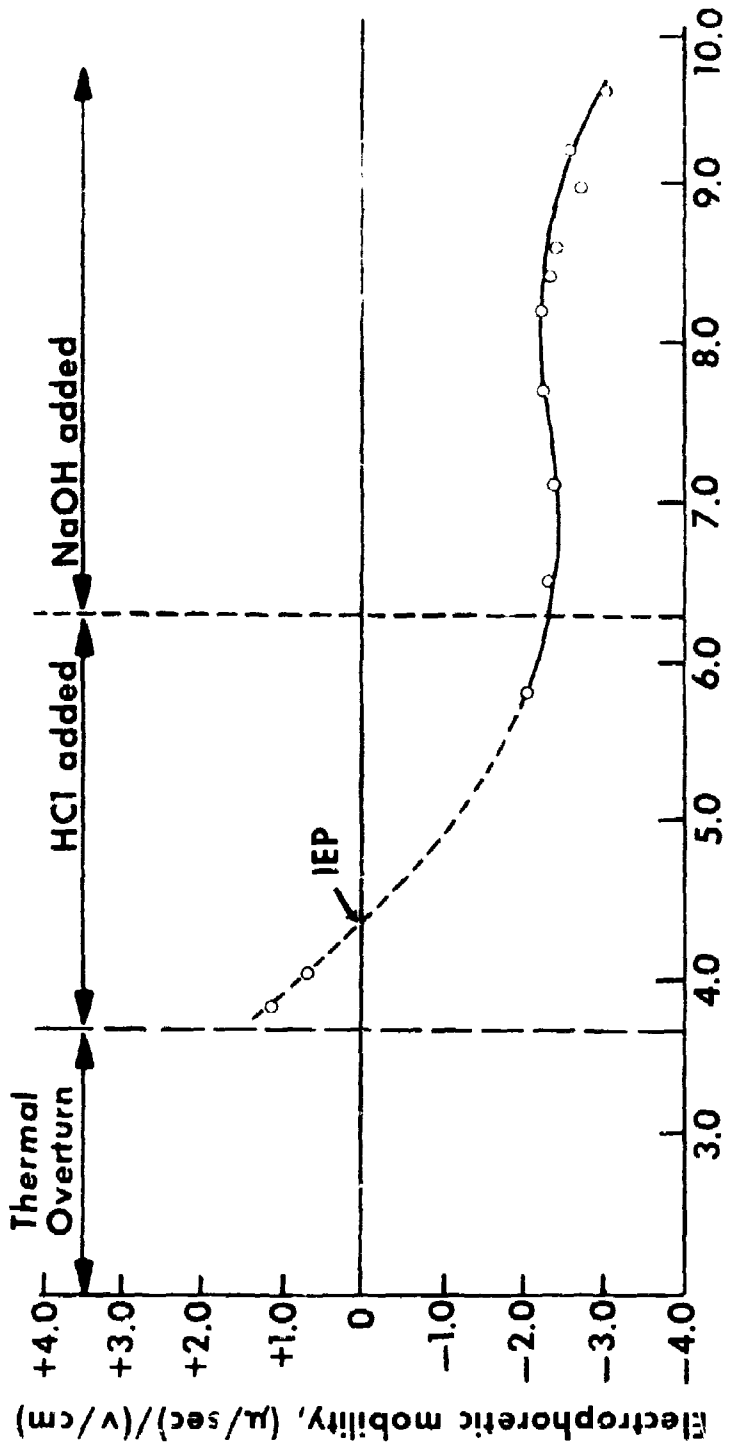

FIGURE 6. Effect of pH on the Stability of a Colloidal System Containing 1 Gm/l Tricalcium Phosphate Medium in Deionized Water Adsorbed on Bentonite Carrier Particles (Weight 1,000 ppm).

in a positive direction, and above this pH, particles migrated in a negative direction. Below pH 3.0, thermal convection occurred, and the SC of the system rose rapidly.

Al_2O_3 particles (1,000 ppm) in deionized water, with tryptose-phosphate medium added at 1% (v/v) to make an anionic colloidal system, were tested to observe the effect of increasing concentrations of various polycationic additives. Results in Figure 7 indicate that a reversal of EM occurs with the addition of Primafloc C-3 and C-7,* Barquat SB-25,** and lead nitrate, but not with the addition of calcium chloride. Calcium chloride and lead nitrate produced extremely high SC values at 1% (v/v) addition levels. Primafloc C-3 produced an alkaline system on increased addition but C-7 produced an acid condition (indicating that the EM reversal in these cases was additive-dependent rather than pH-dependent).

B. ELECTROPHORETIC CHARACTERISTICS OF POLYSTYRENE LATEX CARRIER PARTICLES

Latex particles (1.099 μ diameter) were added to deionized water in the microscope electrophoresis cell in increasing concentration. Visual observations indicated 1 ppm could be used as a carrier system; concentrations above 100 ppm were much too concentrated for optimum viewing. It could not be established that a reversal of EM occurred as a function of concentration of latex particles; at 1, 10, and 100 ppm, the EM was established between -2.0 and -2.5 $(\mu/sec)/(v/cm)$.

Latex particles (1 ppm) in deionized water, with pH adjusted with dilute hydrochloric acid or sodium hydroxide, migrate as shown in Figure 8. In the neutral pH range, the system is anionic; below pH 4.0, it moves through an IEP and becomes cationic.

Tryptose-phosphate medium was added in increasing concentration to the normally anionic, latex-carrier system (10 ppm) at pH 5.8. Figure 9 indicates that the latex carrier particles continue to migrate negatively with the addition of the anionic, hydrophilic medium as would be expected.

The normally anionic, latex-carrier particles (1 ppm suspension) were coated by 0.001 and 1.0% (v/v) additions of hydrophilic colloid (tryptose-phosphate medium), and the system was adjusted to show the EM effect between pH 3.0 and 8.5. Two mobility curves were obtained with a nearly identical IEP between pH 3.0 and 3.3 (Figure 10). There is a possibility that the 0.001% (v/v) addition of hydrophilic colloid did not fully coat the latex particles.

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* Water-soluble, acid cationic polyelectrolyte, Rohm & Haas Co., Independence Mall West, Philadelphia, Pa., 19105.

** Stearyl dimethyl benzyl ammonium chloride, Baird Chemical Industries, Inc., 185 Madison Avenue, New York, N.Y., 10016.

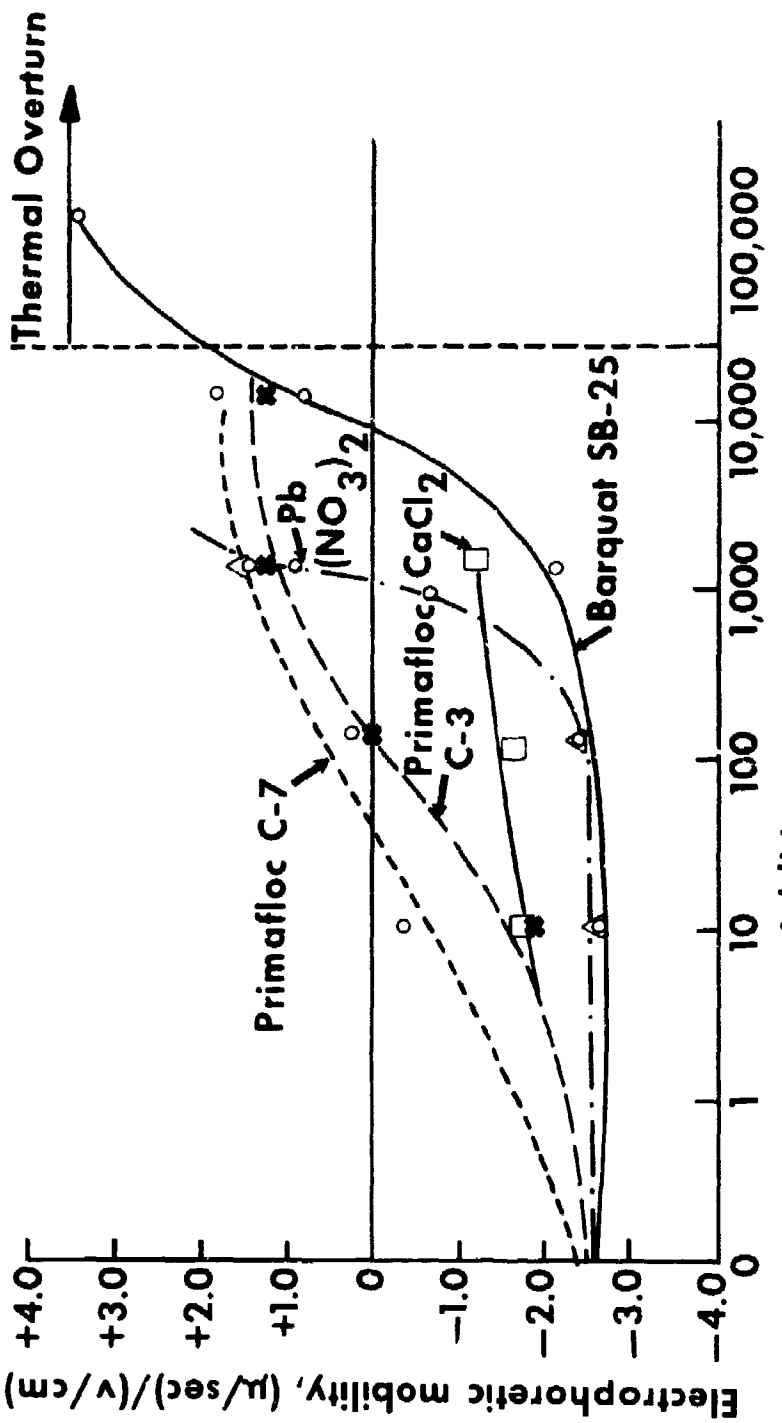

FIGURE 7. Effect of Increasing Concentrations of Five Cationic Additives on the Mobilities of Colloidal Suspensions of 1.0 (w/v) Tryptose-Phosphate Broth in Deionized Water Adsorbed on Polystyrene Latex Particles (Al_2O_3; 1,000 ppm).

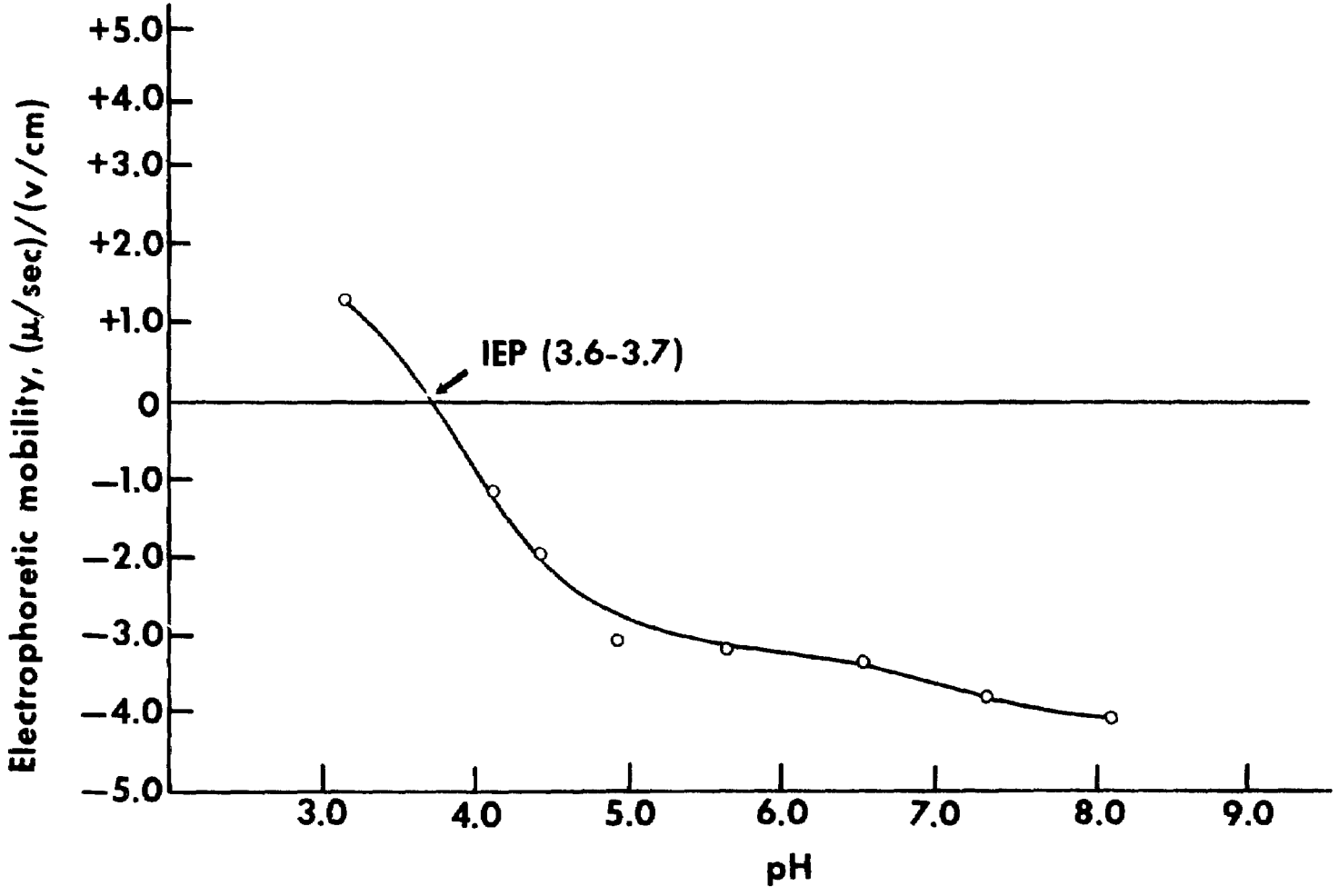

FIGURE 7. Effect of pH on the Mobility of Latex Carrier Particles (1 ppm) in Deionized Water.

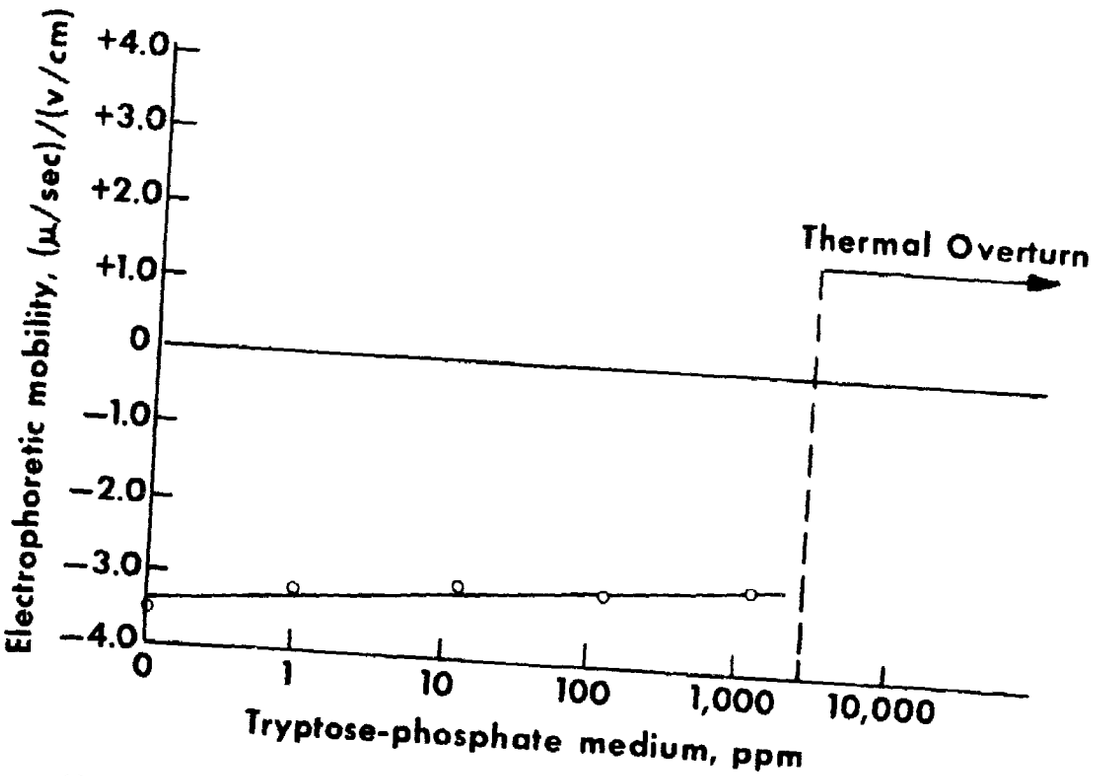

FIGURE 9. Effect of Increasing Concentrations of Tryptose-Phosphate Medium on the Mobility of Latex Carrier Particles (10 ppm) in Deionized Water (pH 5.8).

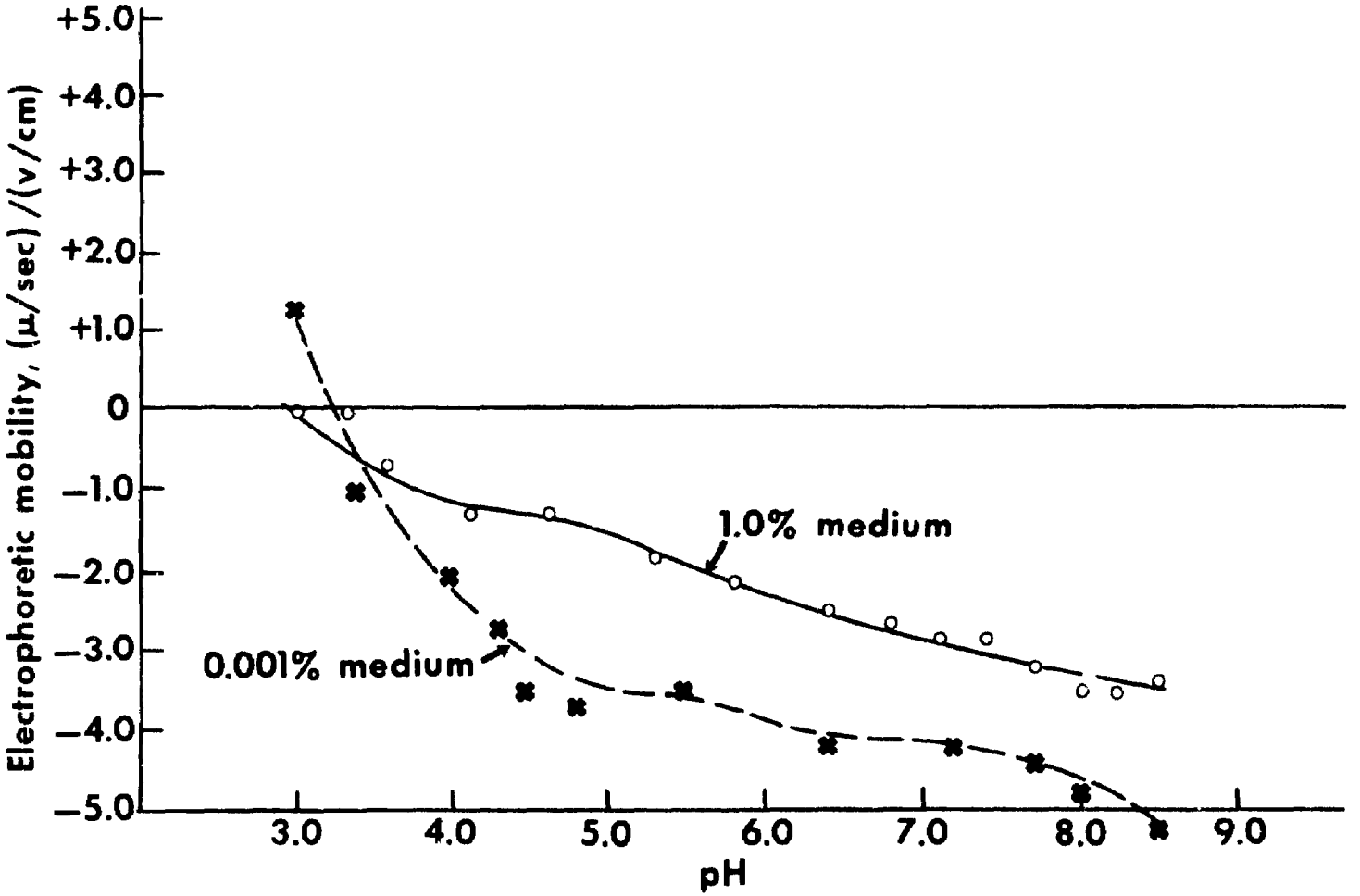

FIGURE 10. Effect of pH on the Mobility of Two Colloidal Systems Consisting of Two Concentrations of Tryptose-Phosphate Medium Adsorbed on Latex Carrier Particles (1 ppm).

A 2% stock solution of the cationic polyelectrolyte, Primafloc C-7, was added in increasing concentrations, up to a maximum of 10 ppm (v/v), to a suspension of 1 ppm latex carrier particles in deionized water. The Primafloc reversed the migration of the latex particles: the EM changed from -3.0 to +1.0 (μ/sec)/(v/cm). The subsequent addition of increasing concentrations of tryptose-phosphate medium (hydrophilic colloid) to the Primafloc-affected system negated the effect of the Primafloc and re-reversed the EM. The data (Fig. 11) demonstrate again the electronegative effect of many medium components, thus underscoring the necessity for purification prior to attempting the EM assay of biocolloids of interest.

Based upon the results comparing the two colloid carrier particles, the polystyrene latex was selected for all further experimentation because (i) the EM of the carrier particle was not concentration-dependent, (ii) the latex was observable at a concentration of 1 ppm, (iii) the particle size distribution of the latex indicated a homogeneous suspension, and (iv) the density of the latex particle was similar to the density of biological particles.

C. ELECTROPHORETIC MOBILITY OF SUBMICROSCOPIC BIOCOLLOIDS ADSORBED ON POLYSTYRENE LATEX CARRIER PARTICLES

Gelatin (acid form)* was added in increasing concentrations to a 1 ppm suspension of latex carrier particles in deionized water at pH 6.5. The average EM for latex without gelatin was -3.1 (μ/sec)/(v/cm); when gelatin was added to only 1 ppm (v/v), migration reversed to +1.2 (μ/sec)/(v/cm) and maintained this mobility at increasingly higher concentrations. A decrease in pH and a slightly increased SC were observed at 1,000 ppm gelatin. Bovine serum albumin and purified staphylococcal enterotoxin B[23] were added to latex carrier suspensions, using the same protocol as for gelatin. Serum albumin did not reverse electromigration but did reduce the migration rate to near -2.0 (μ/sec)/(v/cm). Enterotoxin B (100 ppm) reduced latex mobility to near -1.0 (μ/sec)/(v/cm).

The same three proteins were again adsorbed on 1 ppm latex suspension; pH was adjusted over the range of 2 to 10, and electrophoretic measurements were made for each adsorbed protein preparation. The IEP for acid-form gelatin was established at pH 9.4 to 9.6, for serum albumin at 4.8 to 5.0, and for enterotoxin at 6.3 to 6.4 (Fig. 12, 13 and 14). The IEP for gelatin and serum albumin correspond well with literature values;[14,24] the enterotoxin B IEP is in disagreement with values obtained by Spero et al.[25] who indicated an IEP for purified enterotoxin B at pH 8.6. However, according to Spero, the enterotoxin should be treated by an ion exchange procedure prior to assay for the IEP, and the IEP reported here is typical for the enterotoxin protein with attached phosphate.**

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* Gelatin, USP, Type A, B.P.; partial acid hydrolysis of collogen from skin, connective tissue and bones. IEP pH 7 to 9.

** Personal communication from L. Spero, Physical Science Division, Fort Detrick.

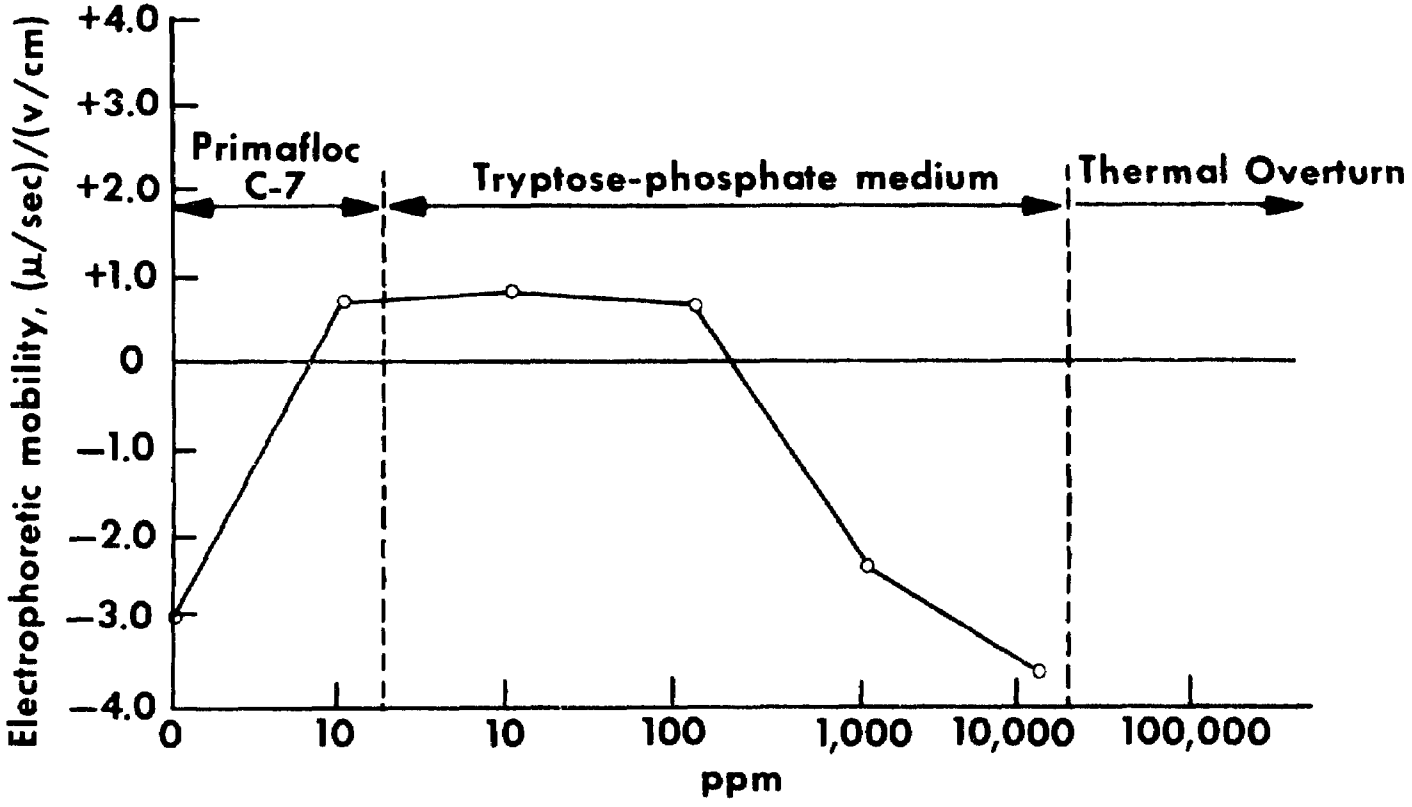

FIGURE 11. Effect of Sequentially Administered Increasing Concentrations of Primafloc C-7 (Cationic) and Tryptose-Phosphate Medium (Anionic) on the Mobility of 1 ppm Latex Carrier Particles in Deionized Water.

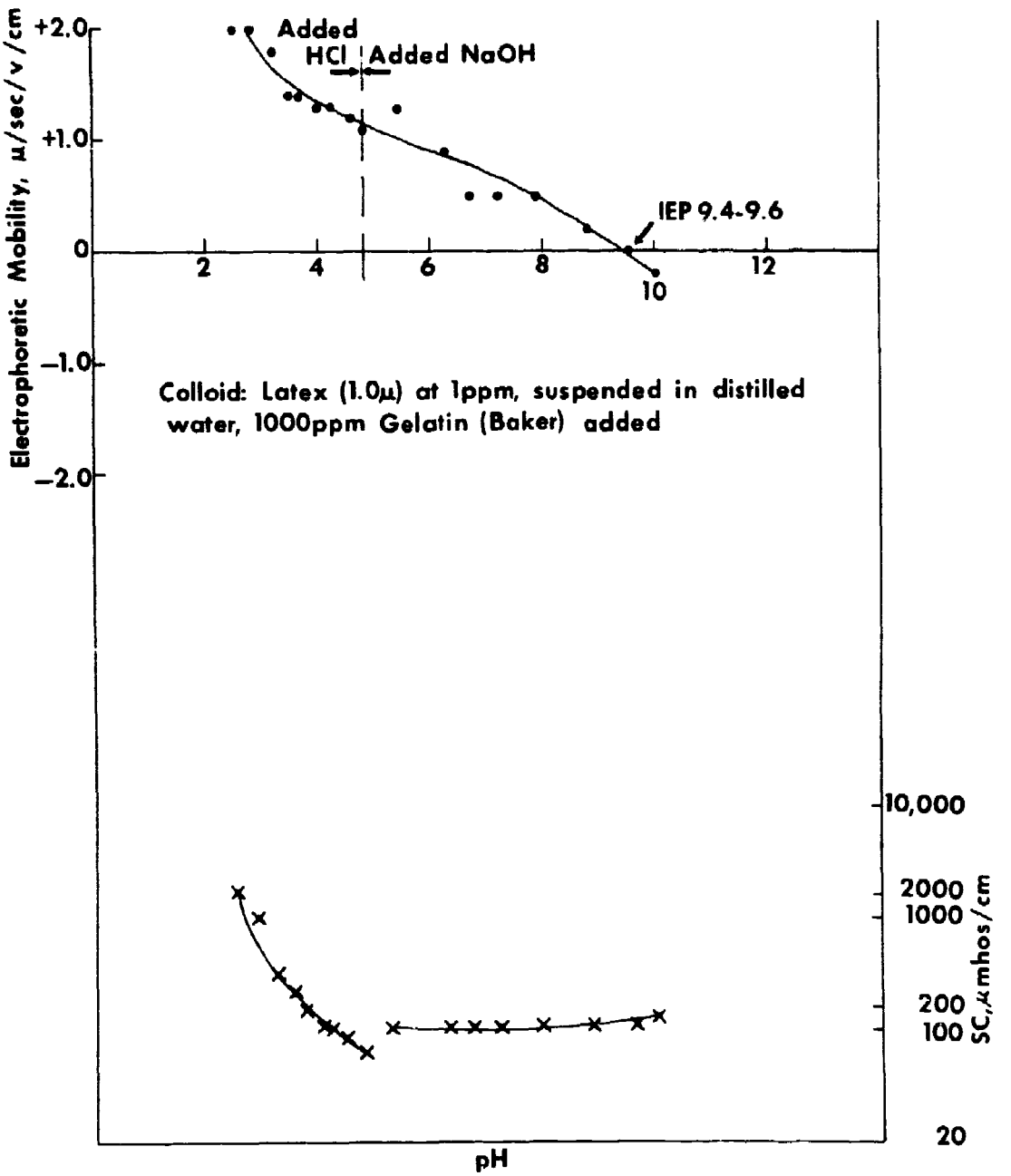

FIGURE 12. Effect of pH on the Mobility and Specific Conductance of a Colloidal System Consisting of 1,000 ppm Acid-Form Gelatin Adsorbed on 1 ppm Polystyrene Latex Particles in Deionized Water.

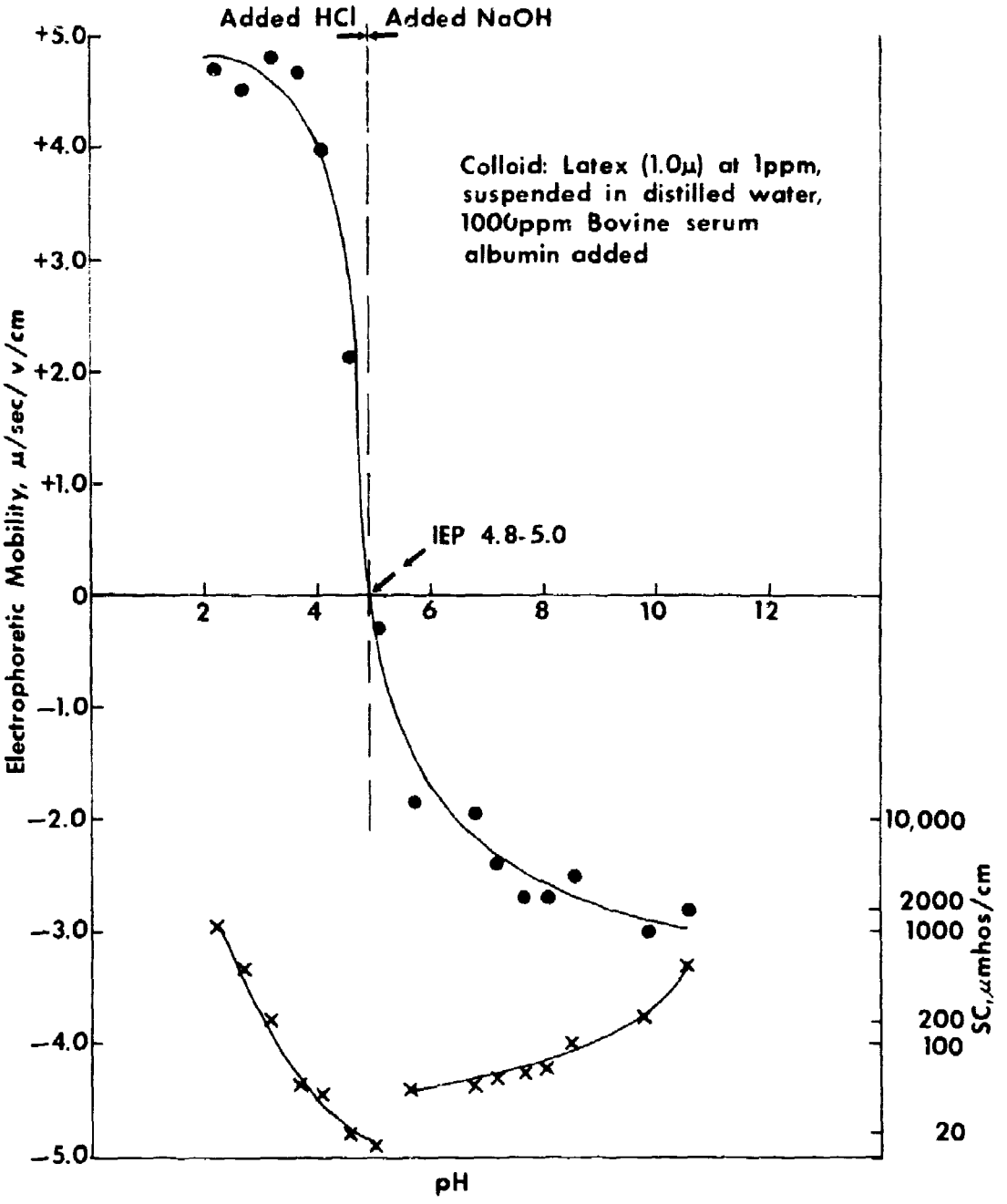

FIGURE 13. Effect of pH on the Mobility and Specific Conductance of a Colloidal System Consisting of 1,000 ppm Bovine Serum Albumin Adsorbed on 1 ppm Polystyrene Latex Particles in Deionized Water.

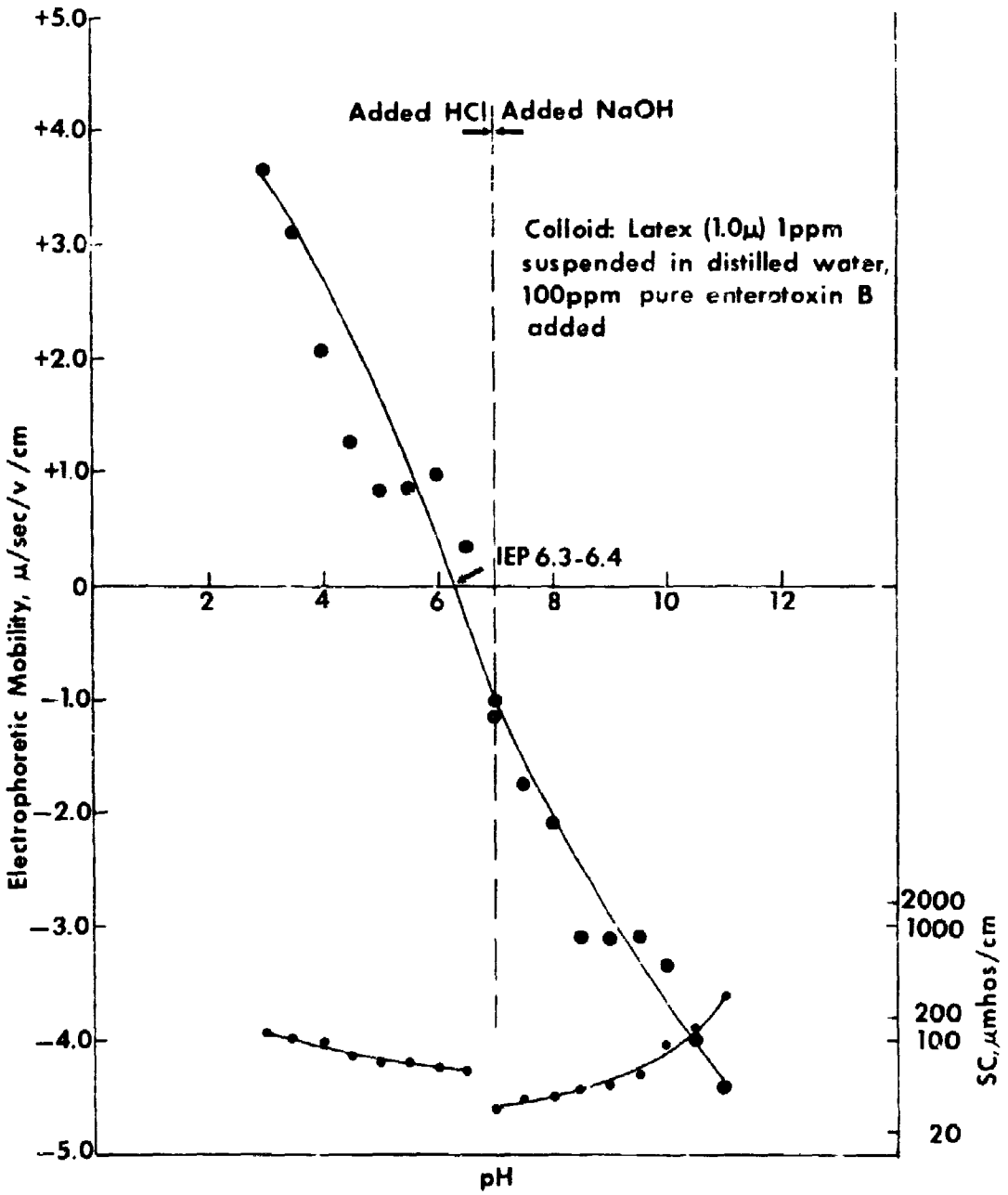

FIGURE 14. Effect of pH on the Mobility and Specific Conductance of a Colloidal System Consisting of 100 ppm Staphylococcal Enterotoxin B Adsorbed on 1 ppm Polystyrene Latex Particles in Deionized Water.

D. ELECTROPHORETIC MOBILITY OF MICROSCOPIC BIOCOLLOIDS

In order to remove the influence of culture media peptone on EM, desorption (dilution) curves were established for E. coli B culture grown in tryptose-phosphate medium. Constant mobilities, ranging between -3.0 and -3.4 (μ/sec)/(v/cm), were recorded at greater than a 1:8 dilution in deionized water. The effect of washing the E. coli cells by resuspension and centrifugation in imidazole buffer or in deionized water resulted in relatively constant EM after the first buffer resuspension.

Wash[a]	EM, (μ/sec)/(v/cm)		SC, μmhos/cm[b]	
	Imidazole	Water	Imidazole	Water
1	-3.0	-3.3	241	155
2	-3.2	-3.1	57	17
3	-3.3	-2.9	51	8
4	-3.7	-2.6	51	7

a. Culture was concentrated at 6,780 x g for 10 minutes and resuspended to original volume in imidazole buffer.

b. Original culture measured 14,000 μmhos/cm SC, and thermal overturn prevented EM measurements.

The effect of pH on the mobility of E. coli B cells is shown in Figure 15; the mobility at pH 7.0 compares with that reported[7] for nonfilamented E. coli B. The IEP for these bacterial cells is at pH 2.8.

S. marcescens, 8UK, bacterial cells had an IEP near pH 2.6 when resuspended in either imidazole buffer or deionized water as shown in Figure 16. The curves of pH versus mobility varied slightly for the two diluents.

Vegetative cells and spores from both an 18-hour culture and a 36-hour autolyzed culture of B. subtilis var. niger were washed twice in deionized water and resuspended in the same diluent for EM assays. EM values for both the cells and spores were similar, and a composite curve is shown in Figure 17. The IEP was projected to pH 2.9 because the SC caused by the addition of acid was too high for mobility measurements above -1.0 (μ/sec)/(v/cm).

S. aureus cells were resuspended in imidazole buffer and examined for the effect of pH; results are shown in Figure 18. The IEP for these cells was projected to pH 2.3 to 2.5.

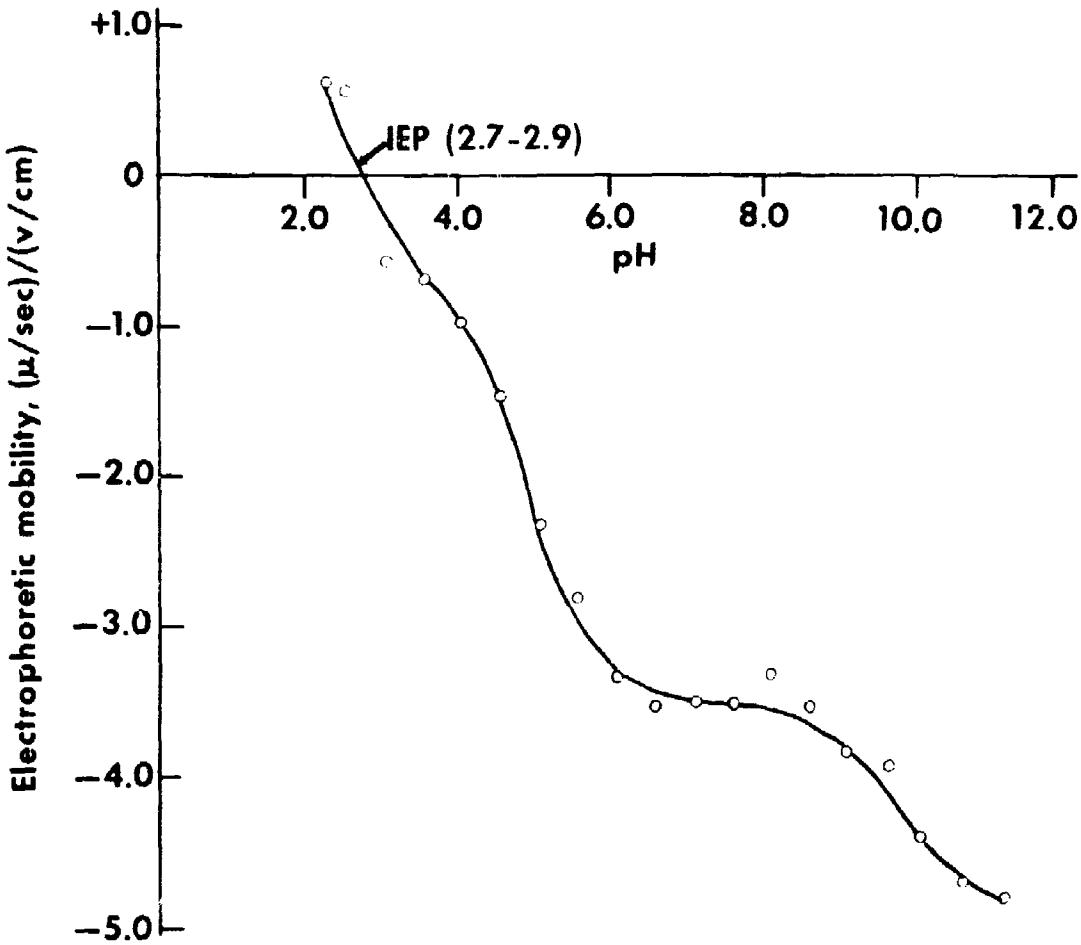

FIGURE 15. Effect of pH on the Mobility of Washed E. coli B Cells Suspended in Deionized Water.

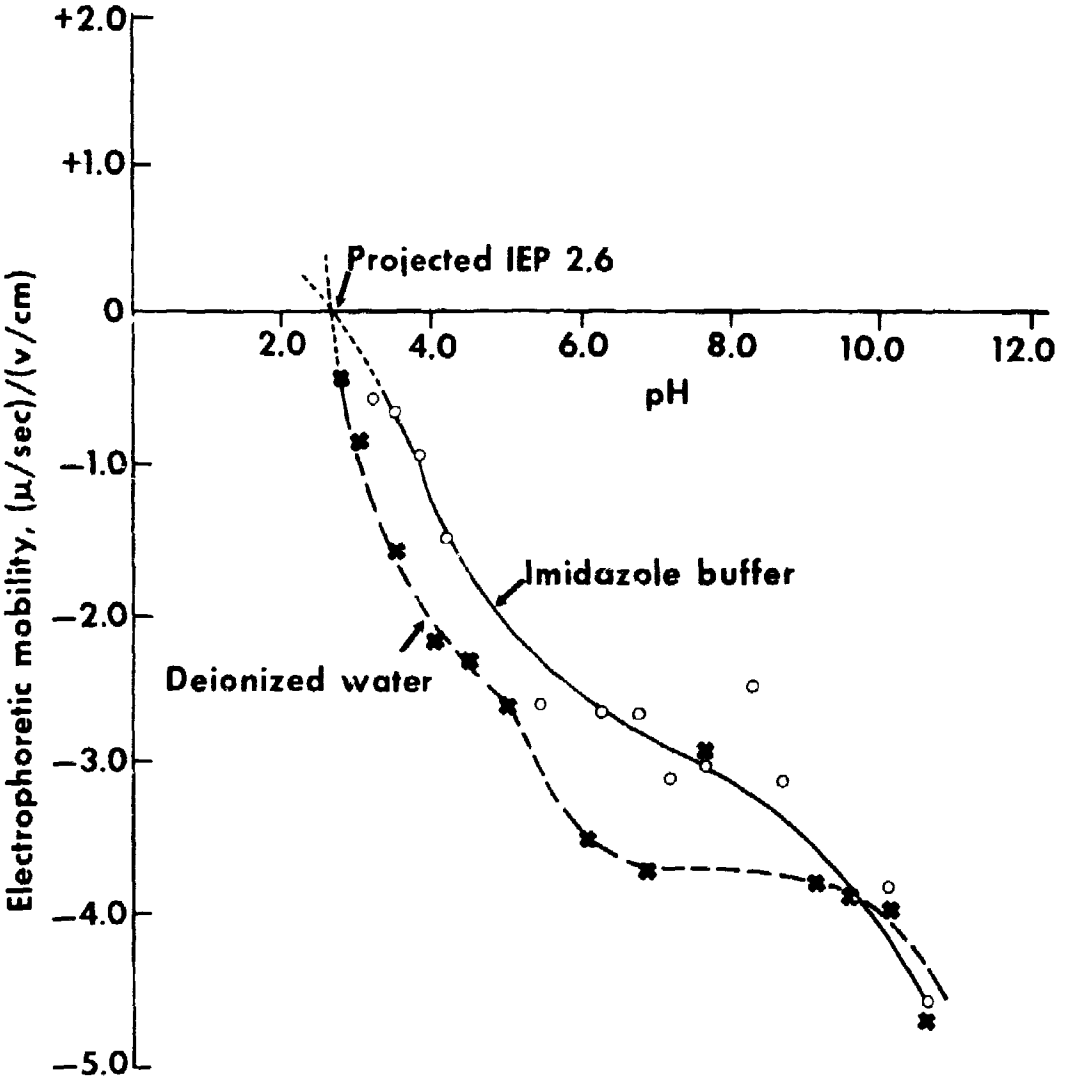

FIGURE 16. Effect of pH on the Mobility of Washed S. marcescens, 8 UK, Cells Suspended in Two Diluents.

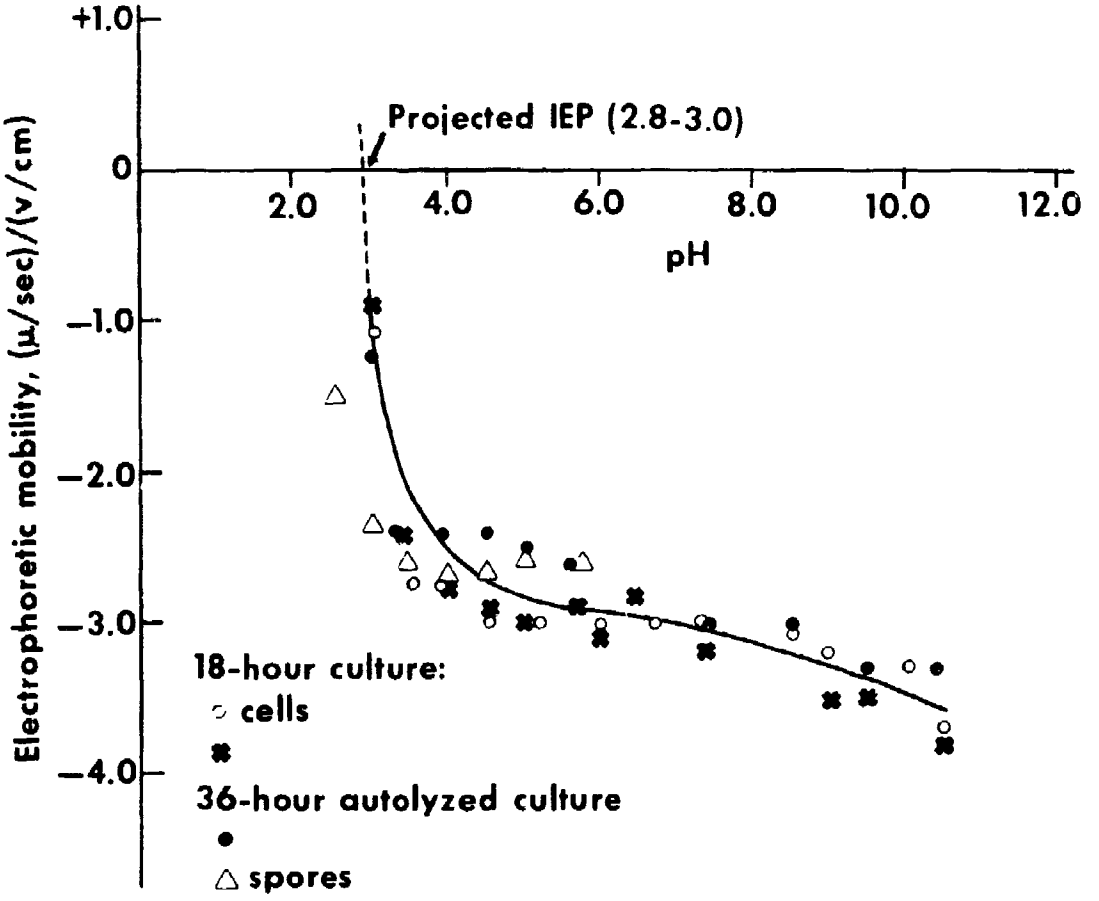

FIGURE 17. Effect of pH on the Mobilities of Four Washed Preparations of B. subtilis var. niger Suspended in Deionized Water.

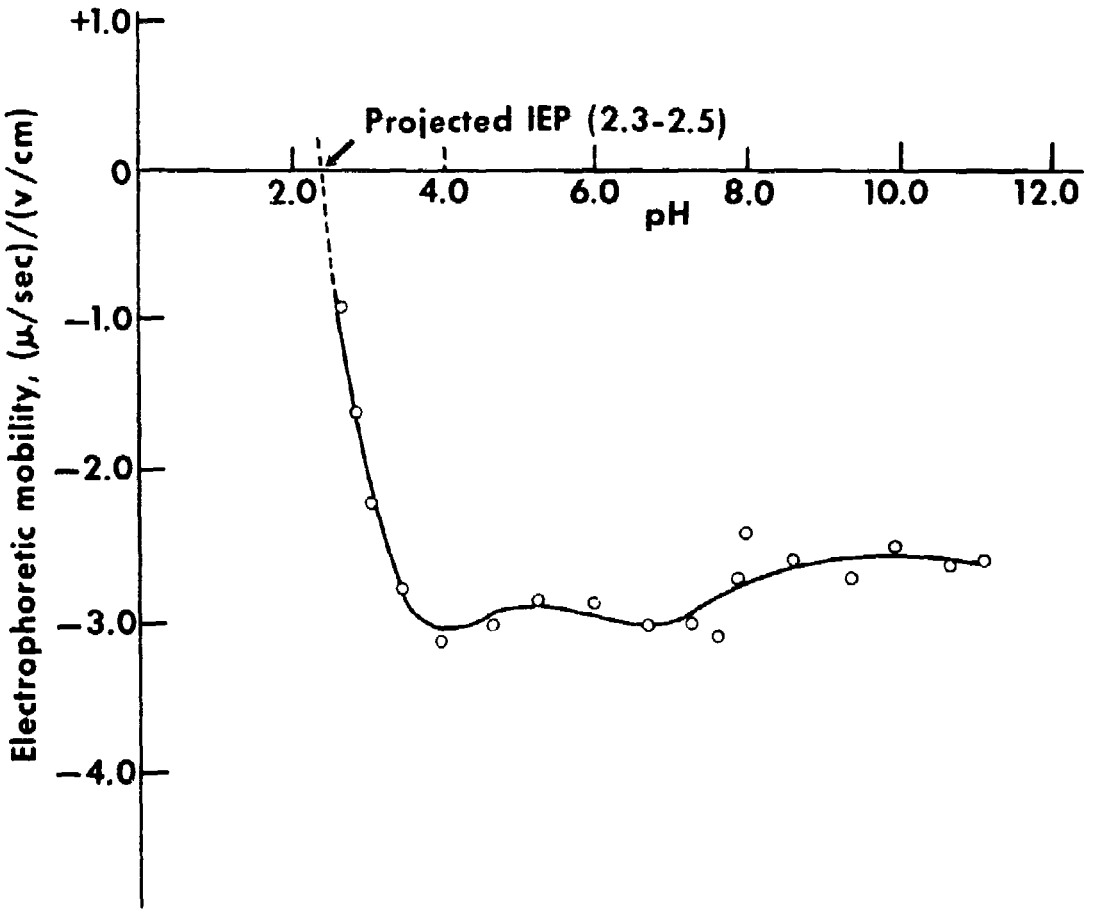

FIGURE 18. Effect of pH on the Mobility of Washed S. aureus, Strain 10-275, Cells Suspended in Imidazole Buffer.

Earle's L strain tissue cells* were diluted 1:8 in 0.32 M sucrose, which reduced the SC from 25,000 to 2,500 μmhos/cm. The effect of washing by resuspension and centrifugation in imidazole buffer plus 0.32 M sucrose or in deionized water plus 0.32 M sucrose resulted in relatively constant EM after either buffer or water resuspension:

Wash[a]	EM, (μ/sec)/(v/cm)		SC, μmhos/cm[b]	
	Imidazole	Water	Imidazole	Water
1	-1.8	-2.0	279	183
2	-2.0	-1.9	41	22
3	-2.0	-1.8	22	9
4	-2.1	-2.0	20	7

a. Centrifuged at 2,500 rpm for 5 min.
b. Original culture SC measured 25,400 μmhos/cm, thermal overturn prevented EM measurement.

The pH versus mobility curve for L cells indicated an IEP near pH 4.4 in 1.3×10^{-6} M imidazole and 0.32 M sucrose buffer as shown in Figure 19.

E. CONTINUOUS PARTICLE ELECTROPHORESIS, PRELIMINARY STUDIES

In order to verify and acquaint ourselves with the operation of the CPED (Fig. 20), we used polystyrene latex spheres of known particle diameter (0.1, 0.3, and 1.1 μ) both as individual homogeneous suspensions and as mixed heterogeneous suspensions for evaluating separation in the CPED. The latex samples were introduced into the CPED at 0.08 ml/min into curtain buffer (0.001 M sodium barbital, pH 7.9) flowing at 20 ml/min. Each sized suspension of latex migrated at increasing voltage, as shown in Figure 21. Mixed latex suspensions (0.1, 0.3, and 1.1 μ) at 30 v/cm matched the values predicted by each sized suspension, indicating that separation occurred as a function of particle size (surface area); a demonstration of particle separation at the viewing area is shown in Figure 22. The separation of the mixture of three sizes (0.1, 0.3, 1.1 μ) of latex particles was further substantiated by examination of the contents of the collected fractions from the CPED:

Collection Tube No.	24	25	26	27	28	29	30
Visible Turbidity	-	+	-	+	-	+	-

Microscopic examination of the collection tube contents verified the separation indicated by turbidity.

* These cells are similar to American Type Culture Collection certified cell line 1.2, L-M, a derivative of NTCC clone 929 (L) connective tissue, mouse.

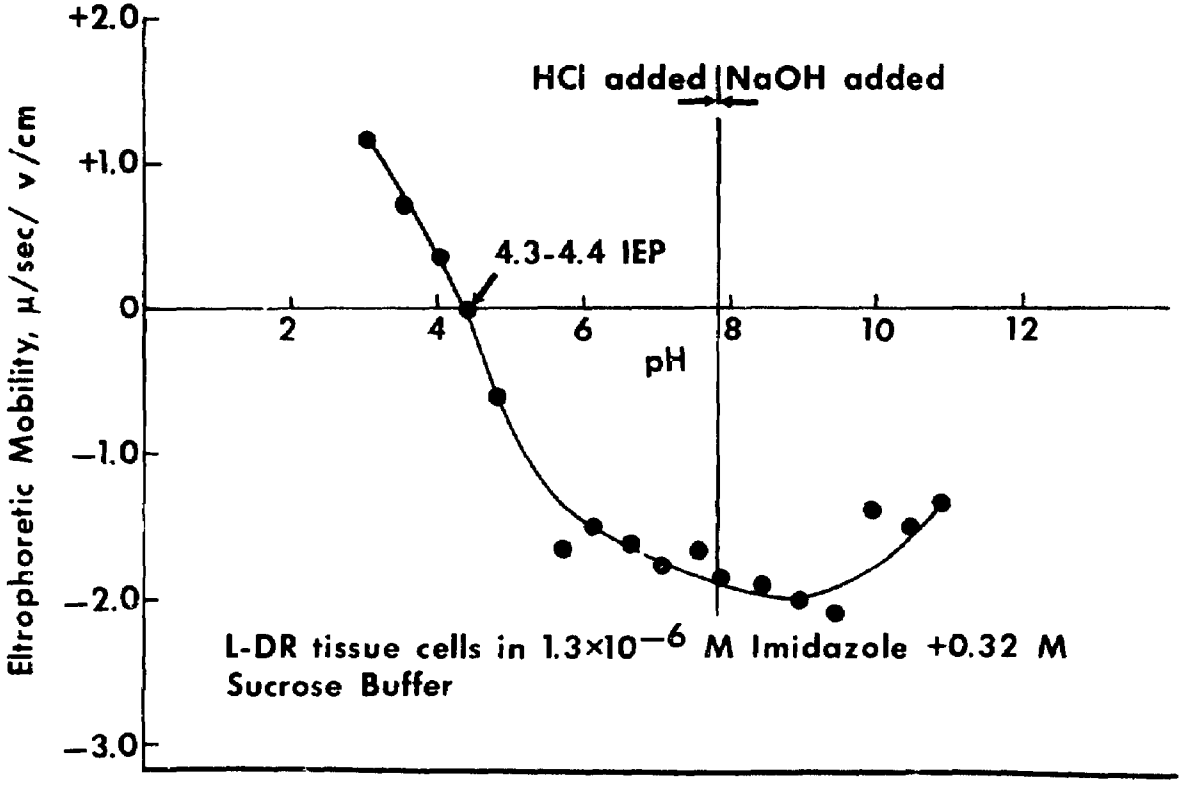

FIGURE 19. Effect of pH on the Mobility of Washed Earle's L Cells (Mouse Fibroblast) Suspended in 1.3×10^{-6} M Imidazole Plus 0.32 M Sucrose Buffer.

FIGURE 20. The Continuous-Flow Particle Electrophoresis Device in Operation.

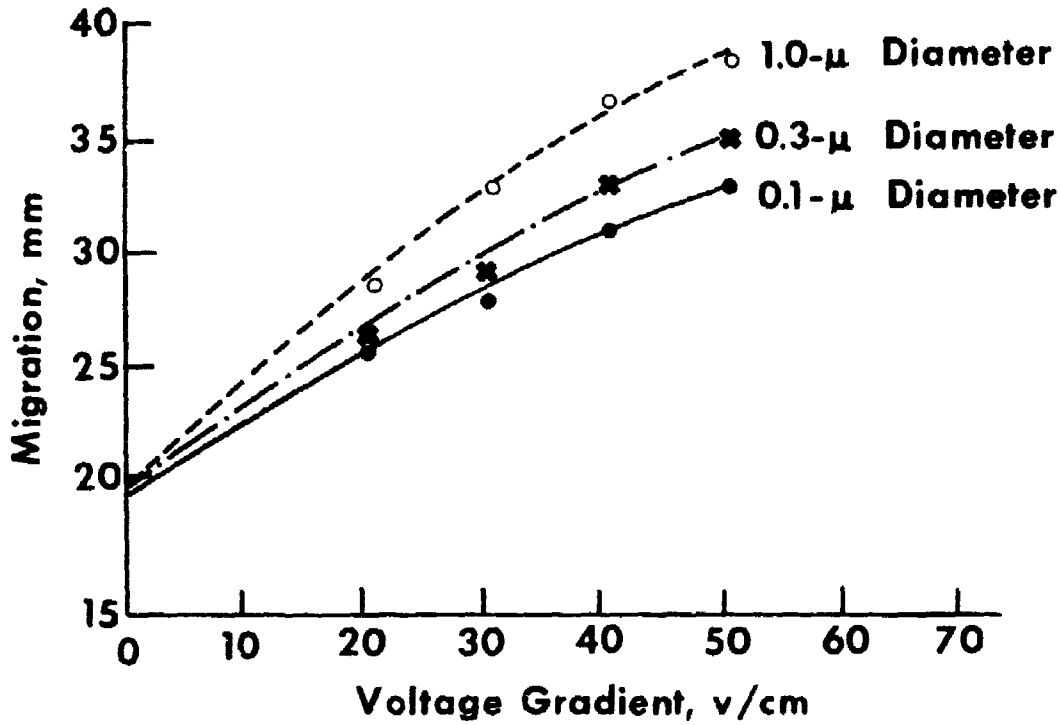

FIGURE 21. Effect of Increased Voltage on the Migration of Three Sizes of Polystyrene Latex Particles Suspended in 0.001 M Sodium Barbital in the Continuous-Flow Particle Electrophoresis Device.

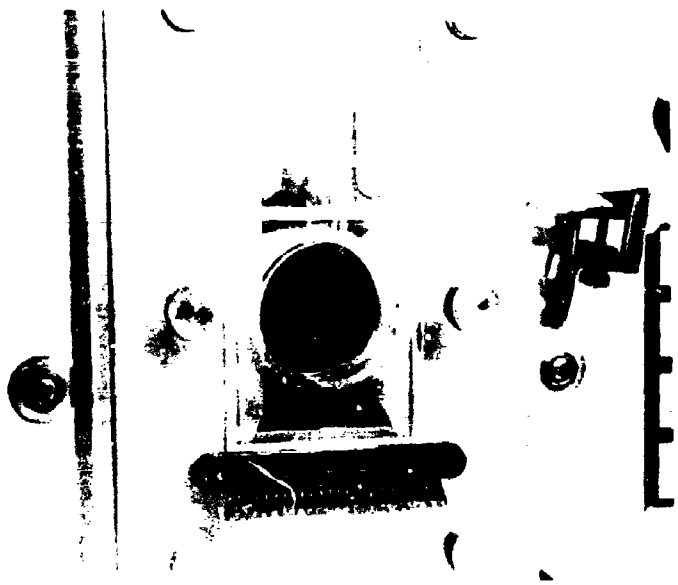

FIGURE 22. Particle Separation at the Viewing Area of the Continuous-Flow Electrophoresis Device.

After the demonstration with latex sphere separation by CPE, we felt that the CPED should be capable of separating mixtures of particles with differing mobilities and of providing samples of electrophoretically separated materials for biological or chemical assay. Subsequently, a partially purified staphylococcal enterotoxin B protein solution was fed into the CPED at 0.08 ml/min, with the buffer curtain (0.001M sodium barbital or 10^{-6} M imidazole at pH 8.6) flow at 20 ml/min, to measure migration and separation at increasing voltage with visible separation results:

	Applied Potential, v/cm						
	0	10	20	30	40	50	75
Barbital Buffer System							
Band position, mm	18	20	21.5	24.5[a]	Thermal overturn		
Δ Mig., mm	0	2	3.5	5.5			
Calc. EM, (μ/sec)/(v/cm)	0	-2.4	-2.4	-3.2			
Imidazole Buffer System							
Band position, mm	19	20	21	21.5[a]	23.5[a]	Thermal	
Δ Mig., mm	0	1	2	2.5	4.5	overturn	
Calc. EM, (μ/sec)/(v/cm)	0	-1.2	-1.4	-1.3	-2.4		

a. The band spread considerably with increased voltage and these values represent the approximate midpoint of the band.

The enterotoxin protein was assayed by the Oudin gel diffusion method with collection tube separation and recovery values in terms of enterotoxin activity:

Voltage, v/cm	No. of Tubes with Enterotoxin	Tube No.	Enterotoxin Recovery, %
	BARBITAL BUFFER SYSTEM		
0	2	15,18	79
10	2	15,18	81
20	3	15,17,18	101
30	4	17,18,19,20	47
	IMIDAZOLE BUFFER SYSTEM		
0	2	15,18	117
10	2	17,18	74
20	2	17,18	73
30	2	17,18	124
40	3	17,18,19	76
50	5	15,17,18,19,20	24

From this information, we noted that the visible band migration indicated nontoxin-particle migration, while the nonvisible but active enterotoxin protein migrated only slightly or not at all at this pH, as indicated by the activity values on assay of the collection tube contents. Based upon the toxin activity and the total solids in the fraction collector samples, the purity of the enterotoxin increased after passage through the CPED at 30 v/cm. The imidazole buffer system performed better than the barbital system and allowed higher voltage to be used before thermal overturn became a problem.

Next, we wanted to demonstrate that microscopic carrier particles could be added to aid in visual identification of band migration of submicroscopic particles in the CPED. Coliphage T-3 lysate with and without added latex particles (1.1 μ; 1 ppm) was introduced into the CPED at 0.04 ml/min into curtain buffer (10^{-6} M imidazole at pH 6.8) flowing at 20 ml/min. The concentration of phage lysate introduced into the CPED made it sufficiently turbid to allow visual comparison of band migration with or without latex, and migration results were in general agreement. Recovery values were based upon individual observations at each applied potential level in terms of PFU out of the indicated number of collection tubes divided by total PFU of phage input to the CPED:

T-3 lysate	Applied Potential, v/cm 0	20	30	40
Band position, mm	18	19.5	26	32
No. of tubes with phage	3	4	3	1
% of phage recovered	74	90	17	<1
T-3 lysate + 1 ppm latex				
Band position, mm	18	20.5	31	34
No. of tubes with phage	2	3	3	6
% of phage recovered	ND	107	71	78

Coliphage T-3 had been recovered from the CPED when adsorbed on 1.1-μ latex particles, and the CPED band position was visible and indicative of phage migration. Coliphage T-3 from lysate was again adsorbed on 1 ppm of 1.1-μ latex particles. The phage samples were introduced into the CPED at a flow rate of 0.04 ml/min; the buffer curtain was flowing at 20 ml/min, the buffer was 10^{-6} M imidazole at pH 7.0 and 25 C, and 30 v/cm were applied. After sufficient time for the phage band to stabilize in position, samples were collected from the CPED fraction collector. The total viable recovery of coliphage (61%) was collected from band positions between 22 and 26 mm, as shown in Figure 23. From the electronegative migration distance, the flow rate, and the applied potential, the EM for coliphage at this pH may be approximated as -3.5 (μ/sec)/(v/cm).

The next series of separations in the CPED were set up as the previous one with coliphage T-3, except that electropositive protein (gelatin) was added to the sample at 100 ppm. The resultant separation within the CPED is also shown in Figure 23. The presence of the gelatin shifted the migration of the phage toward the negative electrode with two maxima, band positions 18.5 and 22.5 mm. The total recovery of phage PFU was 90%.

Four bacteria were selected for electrophoretic separation by CPE. B. subtilis var. niger, E. coli B, S. aureus, and S. marcescens were individually cultured in shake flasks in nutrient medium (Difco). Samples of each culture were individually centrifuged and washed two times in deionized water (pH 7.0) in order to remove adsorbed culture medium protein. Finally, the washed cells were individually resuspended in 10^{-6} M imidazole buffer at three pH levels. Each bacterial suspension was measured in the CPED for particle band migration at 20, 30, and 40 v/cm. Samples at each separation voltage were assayed for viable cell count, pH, and turbidity, as shown in Table 2. The migration distances were converted to EM and plotted versus pH (Fig. 24). Based on these separations by the mobility of the four bacterial cultures, it was expected that a mixture of these bacterial cultures could be separated by CPE in imidazole buffer at pH 7.0 and with 30 v/cm applied. Based upon the CPE data plots of pH versus calculated EM shown in Figure 24, the EM for the individual washed bacterial cultures were estimated for pH 7.0 in 10^{-6} M imidazole buffer: S. marcescens, 1.8 (μ/sec)/(v/cm); B. subtilis var. niger, 3.1 (μ/sec)/(v/cm); S. aureus, 4.2 (μ/sec)/(v/cm); and E. coli B, 4.5 (μ/sec)/(v/cm). These values compare favorably with those reported[7] at the same pH, but in a phosphate buffer of higher ionic strength.

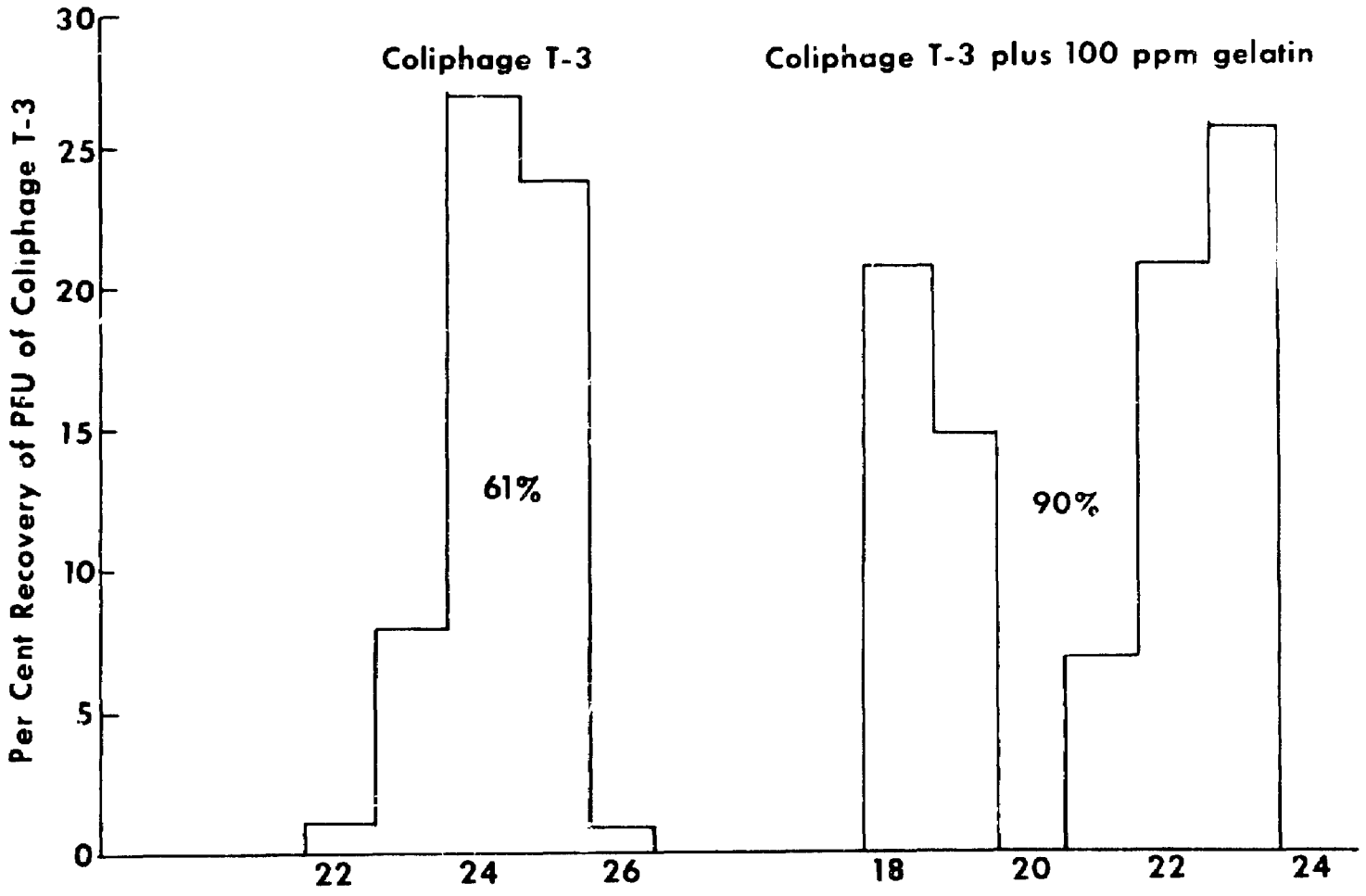

FIGURE 23. Continuous-Flow Particle Electrophoresis: Viable Recovery of Coliphage T-3 Adsorbed on 1 ppm Polystyrene Latex Particles (1.1 μ) with and without Added Gelatin. Conditions: 10^{-6} M imidazole buffer at pH 7.0 and 25 C; 0.04 ml/min product flow; 20 ml/min curtain flow; 30 v/cm applied. Numbers in the histograms indicate total per cent viable recovery.

TABLE 2. ELECTROPHORETIC CHARACTERISTICS OF BACTERIA BY CONTINUOUS PARTICLE ELECTROPHORESIS

pH[a]	Applied Potential, v/cm			
	0	20	30	40
6.6 Bacteria	SM[b]	EC[b]	SA[b]	BG[b]
Band Position, mm	19.5	25.0	26.8	26.5
Δ Mig., mm	0	5.5	7.3	7.0
Calc. EM, (μ/sec)/(v/cm)	0	-4.4	-3.9	-2.8
% Cell Recovery	86	79	61	107
Tube Max. Viability	18	22	21	23
Tube Max. Turbidity	18	21	21	23
7.6 Bacteria	EC	SA	BG	SM
Band Position, mm	19.5	25.3	26.3	26.5
Δ Mig., mm	0	5.8	6.8	6.8
Calc. EM, (μ/sec)/(v/cm)	0	-4.7	-3.6	-2.7
% Cell Recovery	102	15	143	84
Tube Max. Viability	18	23	25	26
Tube Max. Turbidity	18	23	25	27
8.6 Bacteria	SA	BG	SM	EC
Band Position, mm	19.5	27.5	28.5	32.3
Δ Mig., mm	0	8.0	9.0	12.8
Calc. EM, (μ/sec)/(v/cm)	0	-6.4	-4.8	-5.2
% Cell Recovery	85	108	102	96
Tube Max. Viability	17	28	25	34
Tube Max. Turbidity	17	26	25	32

a. 10^{-6} M imidazole buffer.

b. SM, S. marcescens; EC, E. coli; SA, S. aureus; and BG, B. subtilis var. niger.

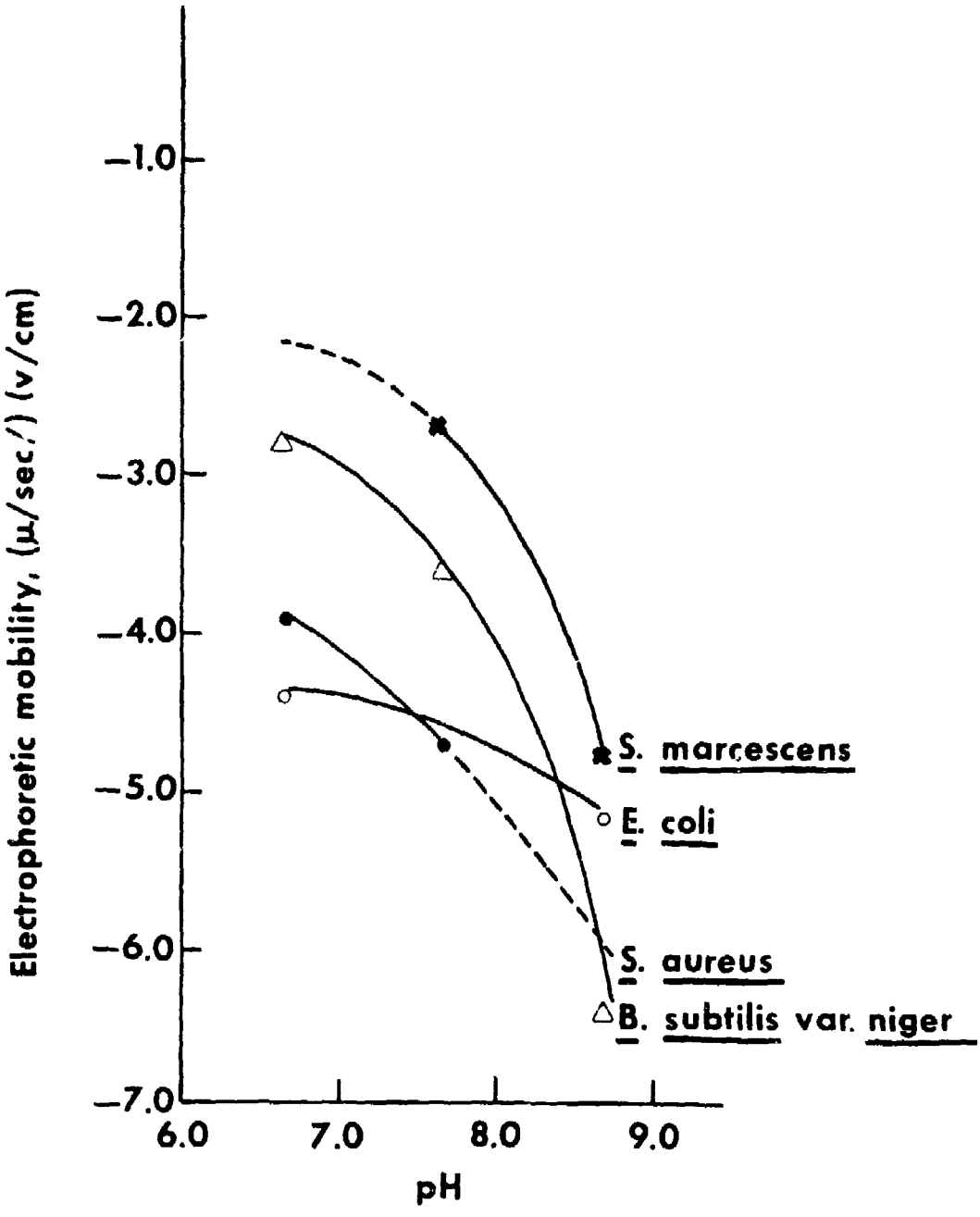

FIGURE 24. Continuous-Flow Particle Electrophoresis: Effect of pH on the Mobility of Individual Suspensions of Four Washed Bacteria in 10^{-6} M Imidazole Buffer.

In order to determine whether the CPED could separate mixed bacteria of similar EM, the four washed bacterial cell suspensions were prepared as before and mixed just prior to introducing the sample into the CPED. The CPE test conditions were: pH 7.0, 10^{-6} M imidazole buffer, temperature controlled to 25 C, sample flow rate 0.04 ml/min, curtain buffer flow at 20 ml/min, and 30 v/cm applied potential. Based upon the EM values for the individual bacteria, the fraction collector tube position was predicted for the maximum collection of each bacterial species. The viable recoveries of each bacterial species from the mixture are shown versus collection tube position in Figure 25. The sample band in the CPED migrated as a single band approximately 2.5 mm wide; separate banding was not distinguishable. The fraction collector tubes indicated turbidity in tubes 21 through 27 with the maximum in tube 24. Because the bacterial bands were not distinguishable, differential plating media were used for the samples from tubes 20 through 28: tellurite agar* for B. subtilis var. niger, EMB agar (Bacto) for S. marcescens and E. coli B, and salt-mannitol agar (Bacto) for S. aureus. It is obvious from the viable recovery maxima for each bacterial suspension (Fig. 25) that we could not differentiate among S. aureus, E. coli B, and B. subtilis var. niger, except by differential plating of the sample from the CPED. S. marcescens may have been differentiated by its migration position, but the CPE samples showed S. marcescens in six tubes. A major loss of viability was observed for S. aureus under the conditions of the test. It is worth noting that the four bacterial species were grown in identical medium prior to washing and that each culture was harvested in the late logarithmic growth phase.

In order to test CPE for the separation of bacteria at higher voltage, S. marcescens, and E. coli B were prepared as before, mixed prior to admission into the CPED, and separation was attempted at 60 v/cm. The E. coli cells migrated to the same position as they had with 30 v/cm, and viable recovery was 57%; S. marcescens viable recovery was only 5%.

In one of our CPE separations of E. coli B cells, two distinct fractions were collected. Huebner[10] reported two visible bands on E. coli separation by CPE and presumed them to be filamented and nonfilamented forms of the bacteria. Brinton and Lauffer[7] report two forms of E. coli with distinctly differing mobilities. We used our 18-hour E. coli B culture grown in nutrient medium (Difco), washed twice and resuspended in 10^{-6} M imidazole buffer to determine if we had E. coli in two EM classes. The sample flow to the CPED was 0.04 ml/min, curtain flow at 22 ml/min, pH 7.0, and 40 v/cm applied potential. There was no indication that this particular culture contained the two types of E. coli cells; the separation results indicated a single band at 27.0 to 29.0 mm, a migration of 8.5 mm, and a calculated EM of -3.0 to -3.4 (μ/sec)/(v/cm), with viable recovery of 98% as shown in Figure 26.

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* Tellurite agar: Bacto tryptose 20 g/liter, dextrose 10 g/liter, NaCl 5 g/liter, Bacto-agar 20 g/liter plus 5 ml 1% potassium tellurite stock solution.

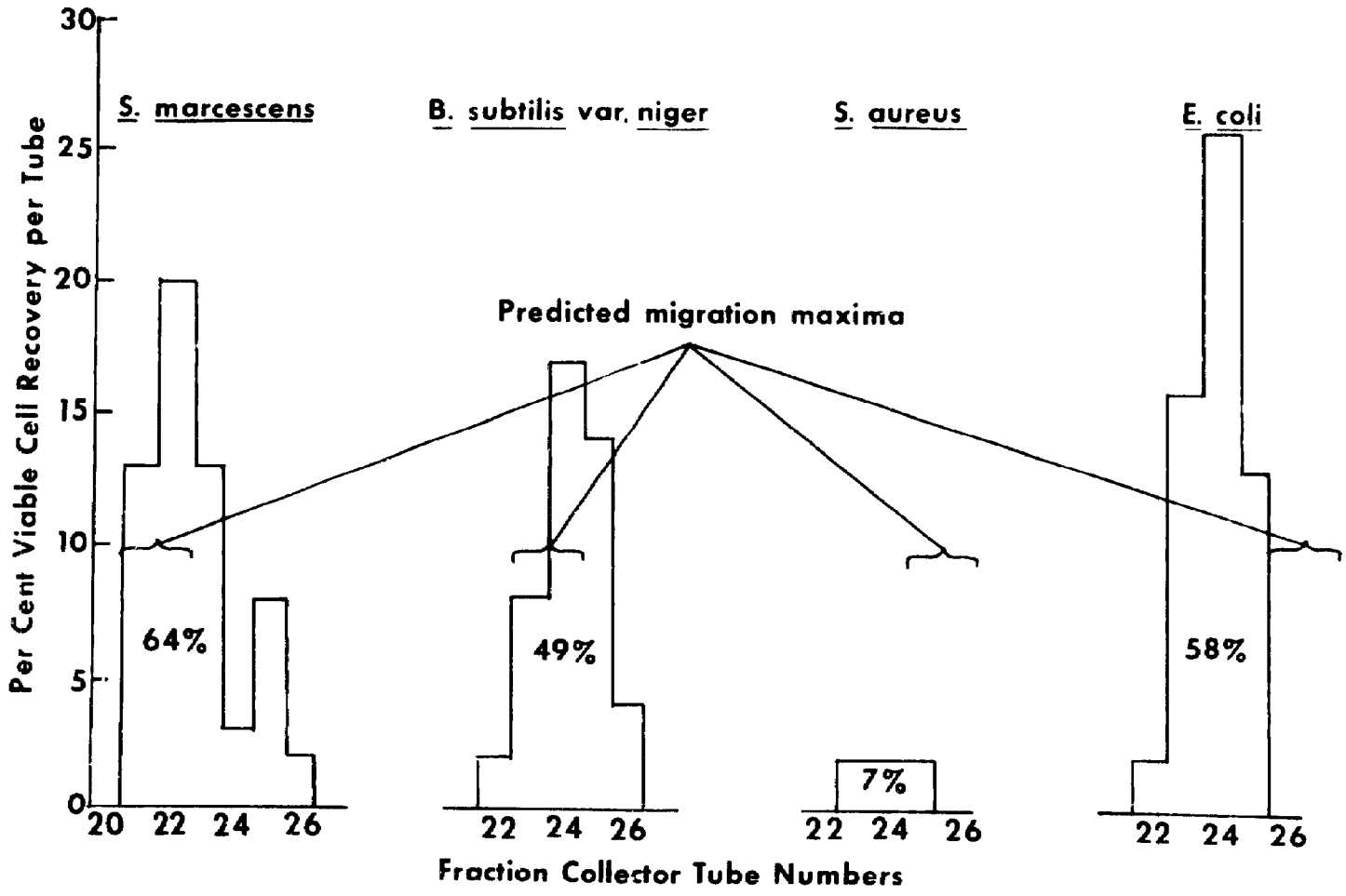

FIGURE 25. Continuous-Flow Particle Electrophoresis: Viable Recovery of Four Bacteria from a Heterogeneous Mixture of Washed Cells and Assayed on Differential Media. Conditions: 10^{-6} M imidazole buffer at pH 7.0 and 25 C; 0.04 ml/min product flow; 20 ml/min curtain flow; 30 v/cm applied. Numbers in the histograms indicate total per cent viable recovery.

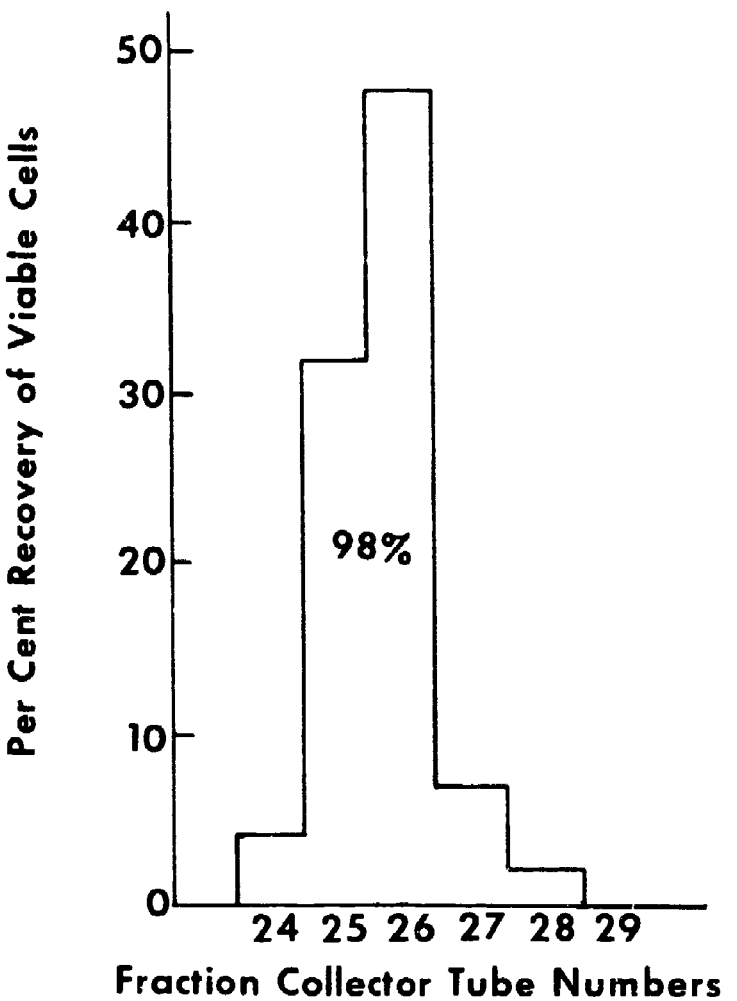

FIGURE 26. Continuous-Flow Particle Electrophoresis: Viable Recovery of Washed E. coli B. Conditions: 10^{-5} M imidazole buffer at pH 7.0 and 25 C; 0.04 ml/min product flow; 22 ml/min curtain flow; 40 v/cm applied. Number in the histogram indicates total per cent viable recovery.

V. CONCLUSIONS

We have demonstrated techniques for the measurement of electrophoretic mobility of biocolloids in two analytical classes, those that are microscopically observable and those that are not directly observable through the microscope.

Particulates that are microscopically observable (i.e., larger than 0.3 μ median diameter) are centrifuged at 6,780 x g for 10 min, resuspended in 10^{-6} M imidazole buffer, recentrifuged and resuspended, with a final centrifugation to compact the doubly washed cells. Sucrose (0.32 M) may be added to the buffer to protect the colloid from osmotic shock. Normally, two complete washes will remove the medium ingredients. A small portion of the compacted cells is resuspended in the buffer for examination of mobility. The pH of the buffer may be adjusted to that level appropriate for the colloid; generally, the pH range used is 6.8 to 8.6. The applied potential for assay in the microscope electrophoresis cell is normally 20 v/cm where SC is less than 700 μmhos/cm. The electrophoretic behavior of these washed colloidal biological materials may be further defined by three measurements: (i) EM and SC at the constant pH required for viability of the biocolloid. (ii) a pH versus mobility plot over the range of pH 2.0 to 10.0 to define the IEP of the colloidal surface, and (iii) where pH variation is not feasible, an electrolyte or polyelectrolyte versus mobility plot over an additive concentration range of 1.0 to 1,000 ppm.

Because dilution of most biocolloids is required either for visibility in the microscope electrophoresis cell or to prevent thermal overturn upon applying voltage, a geometric dilution versus EM plot is required on cell culture to ascertain that EM is not changed more than ±0.5 (μ/sec)/(v/cm) throughout the normal and readable dilution range.

Biological particles that are not directly observable must be adsorbed on the surface of a standard carrier particle that is observable. The carrier particle of choice is polystyrene latex, 1.099 μ diameter, at one ppm (v/v) (of the commercial suspension supplied) in the original colloid sample. The buffer, pH, temperature and EM-related measurements of the carrier-adsorbed colloid are identical to those enumerated for microscopically visible biocolloids. However, it should be emphasized that mobilities of submicroscopic particles are only quantitative when the substance is pure and free from other mobile colloids. Because it is quite difficult to prepare submicroscopic biocolloids completely free from contaminating proteins, any EM measurements on these particulates must be verified by preparative electrophoresis; i.e., in the CPED, where the fractions may be analyzed chemically and biologically.

The CPED appears to be a useful laboratory tool for the separation of electromobile, colloidal biological materials from suspension. The CPED has been utilized for microscopic particles (bacteria) and for submicroscopic particles (coliphage) adsorbed on latex carrier particles.

Quantitative viable recovery of several bacteria and coliphage has been demonstrated in the particle band fractions collected by CPE. The migration and sample position of the bacteria in the CPED correlates well with the EM measured in the microscope electrophoresis cell. The bacterial species from washed suspensions retain their strain-specific mobility provided that the pH, buffer, ionic strength, temperature, and flow rate are held constant. Because of the laminar-flow regime within the CPED, even the motile bacteria may be separated electrophoretically. However, the sample flow during CPE must be controlled with a variable-volume syringe pump to achieve quantitative separation. The useful sample flow of approximately 0.04 ml/min into the curtain buffer flow of about 20 ml/min results in a product fraction dilution of 500-fold. Separation of mixed bacteria has been demonstrated in the CPED when the particle electrophoretic mobilities differ by at least 2 $(\mu/sec)/(v/cm)$ (i.e., S. marcescens cells may be differentiated from E. coli B, S. aureus and B. subtilis var. niger, but the latter three cannot be differentiated from each other by the CPED fraction position unless differential plating media are used).

The electrophoretic behavior of bioparticles is useful in predicting concentration and purification ability for application to sedimentation, flocculation, and filtration operations. Because the precipitation or dispersion of biological suspensions is controlled by forces between the particle surface and the suspending fluid, these separation methods may be enhanced if the colloid is near its IEP (i.e., nondispersed). This microscope electrophoresis technique has been used by the authors, not only to provide the EM and IEP of biocolloids, but also to describe and quantitate flocculants, polyelectrolyte precipitants, or pH necessary to enhance concentration and purification of the biological product. The CPED provides a preparative technique for separation of some colloidal, biological materials. The CPE technique was also useful in demonstrating the separation of coliphage from culture lysate because otherwise unobservable fractions could be collected and identified. The calculated EM determined from the CPED may be influenced by contaminating proteins. However, the technique can be used to determine pH changes or polyelectrolyte additives necessary to shift the EM near the IEP in order to enhance concentration and purification of submicroscopic biological particles by subsequent sedimentation, flocculation or filtration.

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13. ABSTRACT

Biological particles in liquid suspension exhibit varied electrophoretic mobility dependent upon the electric charge behavior of their surface constituents. The surface composition of a population of the same kind of biological particles is uniform in a constant environment. The microscope electrophoresis techniques for mobility and isoelectric point determinations of microscopic particles (bacteria, suspended mammalian tissue cells, aluminum oxide particles, and polystyrene latex particles) and submicroscopic particles (proteins and gelatin) are described. The information that can be obtained and the additives for modification of electrophoretic mobility determined by the analytical method are applied to the preparative, laminar-flow, continuous particle electrophoresis system. Separation of bacteria and bacteriophage from suspensions by continuous particle electrophoresis is described.

14. Key Words

Microscope electrophoresis
Electrophoresis
Mobility curve
Polystyrene latex
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